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UTILITY PATENT APPLICATION TRANSMITTAL <i>(Only for new nonprovisional applications under 37 CFR 1.53(b))</i>	Attorney Docket No. B01927011
	First Named Inventor or Application Identifier GORDON et al.
	Express Mail Label No. EL024661737US
Date of Deposit December 21, 1999	

APPLICATION ELEMENTS <i>See MPEP chapter 600 concerning utility patent application contents</i>	ADDRESS TO: Assistant Commissioner for Patents Box Patent Application Washington, DC 20231
1. <input checked="" type="checkbox"/> Fee Transmittal Form <i>(Submit an original, and a duplicate for fee processing)</i> 2. <input checked="" type="checkbox"/> Application [Total pages: 120] 55 pages specification (inc. 4 pgs. Seq. Listing) 1 page abstract 11 pages claims 94 claims 3. <input checked="" type="checkbox"/> Drawing(s) (35 USC 113) [Total sheets: 53] <input type="checkbox"/> Informal <input checked="" type="checkbox"/> Formal [Total drawings: 35] 4. <input type="checkbox"/> Oath or Declaration [Total pages] a. <input type="checkbox"/> Newly executed (original or copy) b. <input type="checkbox"/> Copy from a prior application (37 CFR 1.63(d)) <i>(for continuation/divisional with Box 17 completed)</i> [Note Box 5 below] i. <input type="checkbox"/> DELETION OF INVENTOR(S) Signed statement attached deleting inventor(s) named in the prior application, see 37 CFR 1.63(d)(2) and 1.33(b). 5. <input type="checkbox"/> Incorporation by Reference <i>(usable if Box 4b is checked)</i> The entire disclosure of the prior application, from which a copy of the oath or declaration is supplied under Box 4b, is considered as being part of the disclosure of the accompanying application and is hereby incorporated by reference therein.	6. <input type="checkbox"/> Microfiche Computer Program (Appendix) 7. <input checked="" type="checkbox"/> Nucleotide and/or Amino Acid Sequence Submission (if applicable, all necessary) a. <input type="checkbox"/> Computer Readable Copy b. <input type="checkbox"/> Paper Copy (identical to computer copy) c. <input type="checkbox"/> Statement verifying identity of above copies ACCOMPANYING APPLICATION PARTS 8. <input type="checkbox"/> Assignment Papers (cover sheet & documents(s)) 9. <input type="checkbox"/> 37 CFR 3.73(b) Statement <input type="checkbox"/> Power of Attorney <i>(when there is an assignee)</i> 10. <input type="checkbox"/> English Translation of Document <i>(if applicable)</i> 11. <input type="checkbox"/> Information Disclosure <input type="checkbox"/> Copies of IDS Statement (IDS)/PTO-1449 Citations 12. <input type="checkbox"/> Preliminary Amendment 13. <input checked="" type="checkbox"/> Return Receipt Postcard (MPEP 503) <i>(Should be specifically itemized)</i> 14. <input type="checkbox"/> Small Entity <input type="checkbox"/> Statement filed in prior Statement(s) application, Status still proper and desired 15. <input type="checkbox"/> Certified Copy of Priority Document(s) <i>(if foreign priority is claimed)</i>
16. Other:	
17. If a CONTINUING APPLICATION , check appropriate box and supply the requisite information: <input type="checkbox"/> Continuation <input type="checkbox"/> Divisional <input type="checkbox"/> Continuation-in-part (CIP) of prior application No.: <input type="checkbox"/> Cancel in this application original claims of the prior application before calculating the filing fee. <input type="checkbox"/> Amend the specification by inserting before the first line the sentence: This application is a <input type="checkbox"/> continuation <input type="checkbox"/> divisional of application serial no. , filed , entitled , and now .	

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For: **VASCULAR ENDOTHELIAL GROWTH FACTOR-X**☐ **DUPLICATE**

Fee Calculation Sheet

CLAIMS	FOR	NUMBER FILED	NUMBER EXTRA	RATE	FEE
	TOTAL CLAIMS (37 CFR 1.16(c))	94-20=	74 x	\$18	= \$ 1332.00
	INDEPENDENT CLAIMS (37 CFR 1.16(b))	12-3=	9x	\$78	= \$ 702.00
	MULTIPLE DEPENDENT CLAIMS (if applicable) (37 CFR 1.16(d)) +			\$260.00	= \$ 260.00
				BASIC FEE (37 CFR 1.16(a))	\$ 760.00
	Total of above Calculations =				\$ 3054.00
	Reduction by 50% for filing by small entity (Note 37 CFR 1.9, 1.27, 1.28).				\$ 0.00
	Assignment Recordation Fee (if any)				\$ 0.00
	Other Fees (e.g., Petition for Extension of Time), if any NOTE: Enter small-entity amount if applicable.				\$ 0.00
	TOTAL =				\$3054.00

1. A check in the amount of \$3054.00 is enclosed.

General Authorization to Charge Deposit Account and General Request for Extension of Time

2. a. ☒ If the filing of any paper in this application necessitates the payment of a fee under 37 CFR \S X1.16 X1.17 or X1.18, and the fee due is in an amount different from any enclosed check or if no check is enclosed, the Commissioner is hereby authorized to charge any deficiency or credit any overpayment to Deposit Account No. 23/2825.
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Separator Sheet



AAA

Application Text

Place Papers behind this sheet in the following order:

- Amendment**
- (English Language Only)**

VASCULAR ENDOTHELIAL GROWTH FACTOR-X

The present invention is concerned with a novel
vascular endothelial growth factor (VEGF) herein
5 designated "VEGF-X", and characterisation of the
nucleic acid and amino acid sequences of VEGF-X.

Introduction

10 Angiogenesis involves formation and proliferation of
new blood vessels, and is an essential physiological
process for normal growth and development of tissues
in, for example, embryonic development, tissue
regeneration and organ and tissue repair.
15 Angiogenesis also features in the growth of human
cancers which require continuous stimulation of blood
vessel growth. Abnormal angiogenesis is associated
with other diseases such as rheumatoid arthritis
psoriasis and diabetic retinopathy.
20 Capillary vessels consist of endothelial cells which
carry the genetic information necessary to proliferate
to form capillary networks. Angiogenic molecules
which can initiate this process have previously been
25 characterised. A highly selective mitogen for
vascular endothelial cells is vascular endothelial
growth factor (VEGF) (Ferrara et al., "Vascular
Endothelial Growth Factor: Basic Biology and Clinical
Implications". Regulation of angiogenesis, by I.D.
30 Goldberg and E.M. Rosen 1997 Birkhauser Verlag
Basle/Switzerland). VEGF is a potent vasoactive
protein which is comprised of a glycosylated cationic
46-49 kd dimer having two 24 kd subunits. It is
inactivated by sulfhydryl reducing agents and is
35 resistant to acidic pH and to heating and binds to
immobilised heparin.

VEGF-A has four different forms of 121, 165, 189 and 206 amino acids respectively due to alternative splicing. VEGF121 and VEGF165 are soluble and are capable of promoting angiogenesis, whereas VEGF189 and VEGF206 are bound to heparin containing proteoglycans in the cell surface. The temporal and spatial expression of VEGF has been correlated with physiological proliferation of the blood vessels (Gajdusek, C.M., and Carbon, S.J., Cell Physiol., 139:570-579, (1989)); McNeil, P.L., Muthukrishnan, L., Warder, E., D'Amore, P.A., J. Cell. Biol., 109:811-822, (1989)). Its high affinity binding sites are localized only on endothelial cells in tissue sections (Jakeman, L.B., et al., Clin. Invest. 89:244-253 (1989)). The growth factor can be isolated from pituitary cells and several tumor cell lines, and has been implicated in some human gliomas (Plate, K.H. Nature 359:845-848, (1992)). The inhibition of VEGF function by anti-VEGF monoclonal antibodies was shown to inhibit tumor growth in immune-deficient mice (Kim, K.J., Nature 362:841-844, (1993)).

VEGF proteins have been described in the following patents and applications all of which are hereby incorporated by reference EP-0,506,477, WO-95/24473, WO-98/28621, WO-90/13649, EP-0,476,983, EP-0,550,296, WO-90/13649, WO-96/26736, WO-96/27007, WO-98/49300, WO-98/36075, WO-98/840124, WO-90/11084, WO-98/24811, WO-98/10071, WO-98/07832, WO-98/02543, WO-97/05250, WO-91/02058, WO-96/39421, WO-96/39515, WO-98/16551.

The present inventors have now identified a further vascular endothelial growth factor, designated herein as "VEGF-X", and the nucleic acid sequence encoding it, which has potentially significant benefits for the treatment of tumours and other conditions mediated by inappropriate angiogenic activity.

Summary of the Invention

In the present application, there is provided a novel
vascular endothelial growth factor, herein designated
5 "VEGF-X", nucleic acid molecules encoding said growth
factor, an expression vector comprising said nucleic
acid molecule, a host cell transformed with said
vector and compounds which inhibit or enhance
angiogenesis. Also provided is the sequence of a CUB
10 domain present in the sequence of VEGF-X which domain
itself prevents angiogenesis and which is used to
treat diseases associated with inappropriate
vascularisation or angiogenesis.

15 Detailed Description of the Invention

Therefore, according to a first aspect of the present
invention there is provided a nucleic acid molecule
encoding a VEGF-X protein or a functional equivalent,
20 fragment, derivative or bioprecursor thereof, said
protein comprising the amino acid sequence from
position 23 to 345 of the amino acid sequence
illustrated in Figure 10. Alternatively, the nucleic
acid molecule of the invention encodes the complete
25 sequence identified in Figure 10 and which
advantageously includes a signal peptide to express
said protein extracellularly. Preferably, the nucleic
acid molecule is a DNA and even more preferably a cDNA
molecule. Preferably, the nucleic acid molecule
30 comprises the nucleotide sequence from position 257 to
1291 of the nucleotide sequence illustrated in Figure
9. In a preferred embodiment the nucleic acid is of
mammalian origin and even more preferably of human
origin.

35

In accordance with the present invention a functional

equivalent should be taken to mean a protein, or a sequence of amino acids that have similar function to the VEGF-X protein of the invention.

5 Also provided by this aspect of the present invention is a nucleic acid molecule such as an antisense molecule capable of hybridising to the nucleic acid molecules according to the invention under high stringency conditions, which conditions would be well
10 known to those skilled in the art.

Stringency of hybridisation as used herein refers to conditions under which polynucleic acids are stable. The stability of hybrids is reflected in the melting
15 temperature (T_m) of the hybrids. T_m can be approximated by the formula:

$$81.5^{\circ}\text{C} + 16.6(\log_{10}[\text{Na}^+] + 0.41 (\% \text{G}\&\text{C}) - 600/l$$

20 wherein l is the length of the hybrids in nucleotides. T_m decreases approximately by 1-1.5°C with every 1% decrease in sequence homology.

The term "stringency" refers to the hybridisation
25 conditions wherein a single-stranded nucleic acid joins with a complementary strand when the purine or pyrimidine bases therein pair with their corresponding base by hydrogen bonding. High stringency conditions favour homologous base pairing whereas low stringency
30 conditions favour non-homologous base pairing.

"Low stringency" conditions comprise, for example, a temperature of about 37°C or less, a formamide concentration of less than about 50%, and a moderate
35 to low salt (SSC) concentration; or, alternatively, a temperature of about 50°C or less, and a moderate to high salt (SSPE) concentration, for example 1M NaCl.

"High stringency" conditions comprise, for example, a temperature of about 42°C or less, a formamide concentration of less than about 20%, and a low salt (SSC) concentration; or, alternatively, a temperature of about 65°C, or less, and a low salt (SSPE) concentration. For example, high stringency conditions comprise hybridization in 0.5 M NaHPO₄, 7% sodium dodecyl sulfate (SDS), 1 mM EDTA at 65°C (Ausubel, F.M. et al. Current Protocols in Molecular Biology, Vol. I, 1989; Green Inc. New York, at 2.10.3).

"SSC" comprises a hybridization and wash solution. A stock 20X SSC solution contains 3M sodium chloride, 0.3M sodium citrate, pH 7.0.

"SSPE" comprises a hybridization and wash solution. A 1X SSPE solution contains 180 mM NaCl, 9mM Na₂HPO₄, and 1 mM EDTA, pH 7.4.

The nucleic acid capable of hybridising to nucleic acid molecules according to the invention will generally be at least 70%, preferably at least 80 or 90% and more preferably at least 95% homologous to the nucleotide sequences according to the invention.

The antisense molecule capable of hybridising to the nucleic acid according to the invention may be used as a probe or as a medicament or may be included in a pharmaceutical composition with a pharmaceutically acceptable carrier, diluent or excipient therefor.

The term "homologous" describes the relationship between different nucleic acid molecules or amino acid sequences wherein said sequences or molecules are related by partial identity or similarity at one or more blocks or regions within said molecules or

sequences.

The present invention also comprises within its scope proteins or polypeptides encoded by the nucleic acid molecules according to the invention or a functional equivalent, derivative or bioprecursor thereof.

Therefore, according to a further aspect of the present invention, there is provided a VEGF-X protein, or a functional equivalent, derivative or bioprecursor thereof, comprising an amino acid sequence from position 23 to 345 of the sequence as illustrated in Figure 10, or alternatively which amino acid sequence comprises the complete sequence of Figure 10. A further aspect of the invention comprises a VEGF-X protein, or a functional equivalent, derivative or bioprecursor thereof, encoded by a nucleic acid molecule according to the invention. Preferably, the VEGF-X protein encoded by said nucleic acid molecule comprises the sequence from position 23 to 345 of the amino acid sequence as illustrated in Figure 10, or which sequence alternatively comprises the sequence of amino acids of Figure 10.

The DNA molecules according to the invention may, advantageously, be included in a suitable expression vector to express VEGF-X encoded therefrom in a suitable host. Incorporation of cloned DNA into a suitable expression vector for subsequent transformation of said cell and subsequent selection of the transformed cells is well known to those skilled in the art as provided in Sambrook et al. (1989), molecular cloning, a laboratory manual, Cold Spring Harbour Laboratory Press.

An expression vector according to the invention includes a vector having a nucleic acid according to

the invention operably linked to regulatory sequences, such as promoter regions, that are capable of effecting expression of said DNA fragments. The term "operably linked" refers to a juxtaposition wherein the components described are in a relationship permitting them to function in their intended manner. Such vectors may be transformed into a suitable host cell to provide for expression of a polypeptide according to the invention. Thus, in a further aspect, the invention provides a process for preparing polypeptides according to the invention which comprises cultivating a host cell, transformed or transfected with an expression vector as described above under conditions to provide for expression by the vector of a coding sequence encoding the polypeptides, and recovering the expressed polypeptides.

The vectors may be, for example, plasmid, virus or phage vectors provided with an origin of replication, and optionally a promoter for the expression of said nucleotide and optionally a regulator of the promoter.

The vectors may contain one or more selectable markers, such as, for example, ampicillin resistance.

Regulatory elements required for expression include promoter sequences to bind RNA polymerase and transcription initiation sequences for ribosome binding. For example, a bacterial expression vector may include a promoter such as the lac promoter and for translation initiation the Shine-Dalgarno sequence and the start codon AUG. Similarly, a eukaryotic expression vector may include a heterologous or homologous promoter for RNA polymerase II, a downstream polyadenylation signal, the start codon AUG, and a termination codon for detachment of the

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ribosome. Such vectors may be obtained commercially or assembled from the sequences described by methods well known in the art.

5 Nucleic acid molecules according to the invention may be inserted into the vectors described in an antisense orientation in order to provide for the production of antisense RNA. Antisense RNA or other antisense nucleic acids may be produced by synthetic means.

10

In accordance with the present invention, a defined nucleic acid includes not only the identical nucleic acid but also any minor base variations including in particular, substitutions in cases which result in a
15 synonymous codon (a different codon specifying the same amino acid residue) due to the degenerate code in conservative amino acid substitutions. The term "nucleic acid sequence" also includes the complementary sequence to any single stranded sequence
20 given regarding base variations.

The present invention also advantageously provides nucleic acid sequences of at least approximately 10 contiguous nucleotides of a nucleic acid according to
25 the invention and preferably from 10 to 50 nucleotides even more preferably, the nucleic acid sequence comprise the sequences illustrated in Figure 3. These sequences may, advantageously be used as probes or primers to initiate replication, or the like. Such
30 nucleic acid sequences may be produced according to techniques well known in the art, such as by recombinant or synthetic means. They may also be used in diagnostic kits or the like for detecting the presence of a nucleic acid according to the invention.
35 These tests generally comprise contacting the probe with the sample under hybridising conditions and detecting for the presence of any duplex or triplex

formation between the probe and any nucleic acid in the sample.

5 The nucleic acid sequences according to this aspect of the present invention comprise the sequences of nucleotides illustrated in Figures 3 and 5.

10 According to the present invention these probes may be anchored to a solid support. Preferably, they are present on an array so that multiple probes can simultaneously hybridize to a single biological sample. The probes can be spotted onto the array or synthesised in situ on the array. (See Lockhart et al., Nature Biotechnology, vol. 14, December 1996
15 "Expression monitoring by hybridisation to high density oligonucleotide arrays". A single array can contain more than 100, 500 or even 1,000 different probes in discrete locations.

20 The nucleic acid sequences, according to the invention may be produced using such recombinant or synthetic means, such as for example using PCR cloning mechanisms which generally involve making a pair of primers, which may be from approximately 10 to 50
25 nucleotides to a region of the gene which is desired to be cloned, bringing the primers into contact with mRNA, cDNA, or genomic DNA from a human cell, performing a polymerase chain reaction under conditions which brings about amplification of the
30 desired region, isolating the amplified region or fragment and recovering the amplified DNA. Generally, such techniques are well known in the art, such as described in Sambrook et al. (Molecular Cloning: a Laboratory Manual, 1989).

35 The nucleic acids or oligonucleotides according to the invention may carry a revealing label. Suitable

labels include radioisotopes such as ^{32}P or ^{35}S , enzyme labels or other protein labels such as biotin or fluorescent markers. Such labels may be added to the nucleic acids or oligonucleotides of the invention and
5 may be detected using known techniques *per se*.

Advantageously, human allelic variants or polymorphisms of the DNA molecule according to the invention may be identified by, for example, probing
10 cDNA or genomic libraries from a range of individuals, for example, from different populations. Furthermore, nucleic acids and probes according to the invention may be used to sequence genomic DNA from patients using techniques well known in the art, such as the
15 Sanger Dideoxy chain termination method, which may, advantageously, ascertain any predisposition of a patient to certain disorders associated with a growth factor according to the invention.

20 The protein according to the invention includes all possible amino acid variants encoded by the nucleic acid molecule according to the invention including a polypeptide encoded by said molecule and having conservative amino acid changes. Conservative amino
25 acid substitution refers to a replacement of one or more amino acids in a protein as identified in Table 1. Proteins or polypeptides according to the invention further include variants of such sequences, including naturally occurring allelic variants which are
30 substantially homologous to said proteins or polypeptides. In this context, substantial homology is regarded as a sequence which has at least 70%, preferably 80 or 90% and preferably 95% amino acid homology with the proteins or polypeptides encoded by
35 the nucleic acid molecules according to the invention. The protein according to the invention may be recombinant, synthetic or naturally occurring, but is

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preferably recombinant.

5 The nucleic acid or protein according to the invention may be used as a medicament or in the preparation of a medicament for treating cancer or other diseases or conditions associated with expression of VEGF-X protein.

10 Advantageously, the nucleic acid molecule or the protein according to the invention may be provided in a pharmaceutical composition together with a pharmacologically acceptable carrier, diluent or excipient therefor.

15 The present invention is further directed to inhibiting VEGF-X *in vivo* by the use of antisense technology. Antisense technology can be used to control gene expression through triple-helix formation of antisense DNA or RNA, both of which methods are
20 based on binding of a polynucleotide to DNA or RNA. For example, the 5' coding portion or the mature DNA sequence, which encodes for the protein of the present invention, is used to design an antisense RNA oligonucleotide of from 10 to 50 base pairs in length.
25 A DNA oligonucleotide is designed to be complementary to a region of the gene involved in transcription (triple-helix - see Lee et al. Nucl. Acids Res., 6:3073 (1979); Cooney et al., Science, 241:456 (1988); and Dervan et al., Science, 251: 1360 (1991), thereby
30 preventing transcription and the production of VEGF-X. The antisense RNA oligonucleotide hybridises to the mRNA *in vivo* and blocks translation of an mRNA molecule into the VEGF-X protein (antisense - Okano, J. Neurochem., 56:560 (1991); Oligodeoxynucleotides as
35 Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988)).

Alternatively, the oligonucleotide described above can be delivered to cells by procedures in the art such that the anti-sense RNA and DNA may be expressed in vivo to inhibit production of VEGF-X in the manner described above.

Antisense constructs to VEGF-X, therefore, may inhibit the angiogenic activity of VEGF-X and prevent the further growth of or even regress solid tumours, since angiogenesis and neovascularization are essential steps in solid tumour growth. These antisense constructs may also be used to treat rheumatoid arthritis, psoriasis and diabetic retinopathy which are all characterized by abnormal angiogenesis.

A further aspect of the invention provides a host cell or organism, transformed or transfected with an expression vector according to the invention. The host cell or organism may advantageously be used in a method of producing VEGF-X, which comprises recovering any expressed VEGF-X from the host or organism transformed or transfected with the expression vector.

According to a further aspect of the invention there is also provided a transgenic cell, tissue or organism comprising a transgene capable of expressing VEGF-X protein according to the invention. The term "transgene capable of expression" as used herein means a suitable nucleic acid sequence which leads to expression of VEGF-X or proteins having the same function and/or activity. The transgene, may include, for example, genomic nucleic acid isolated from human cells or synthetic nucleic acid, including DNA integrated into the genome or in an extrachromosomal state. Preferably, the transgene comprises the nucleic acid sequence encoding the proteins according to the invention as described herein, or a functional

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The invention also further provides a pharmaceutical composition comprising said antibody together with a pharmaceutically acceptable carrier diluent or excipient therefor.

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Proteins which interact with the polypeptide of the invention may be identified by investigating protein-interactions using the two-hybrid vector system first proposed by Chien et al., (1991) Proc. Natl. Acad.

10 Sci. USA 88 : 9578-9582.

This technique is based on functional reconstitution in vivo of a transcription factor which activates a reporter gene. More particularly the technique
15 comprises providing an appropriate host cell with a DNA construct comprising a reporter gene under the control of a promoter regulated by a transcription factor having a DNA binding domain and an activating domain, expressing in the host cell a first hybrid DNA
20 sequence encoding a first fusion of a fragment or all of a nucleic acid sequence according to the invention and either said DNA binding domain or said activating domain of the transcription factor, expressing in the host at least one second hybrid DNA sequence, such as
25 a library or the like, encoding putative binding proteins to be investigated together with the DNA binding or activating domain of the transcription factor which is not incorporated in the first fusion; detecting any binding of the proteins to be
30 investigated with a protein according to the invention by detecting for the presence of any reporter gene product in the host cell; optionally isolating second hybrid DNA sequences encoding the binding protein.

35 An example of such a technique utilises the GAL4 protein in yeast. GAL4 is a transcriptional activator of galactose metabolism in yeast and has a separate

domain for binding to activators upstream of the
galactose metabolising genes as well as a protein
binding domain. Nucleotide vectors may be
constructed, one of which comprises the nucleotide
5 residues encoding the DNA binding domain of GAL4.
These binding domain residues may be fused to a known
protein encoding sequence, such as for example, the
nucleic acids according to the invention. The other
vector comprises the residues encoding the protein
10 binding domain of GAL4. These residues are fused to
residues encoding a test protein. Any interaction
between polypeptides encoded by the nucleic acid
according to the invention and the protein to be
tested leads to transcriptional activation of a
15 reporter molecule in a GAL-4 transcription deficient
yeast cell into which the vectors have been
transformed. Preferably, a reporter molecule such as
 β -galactosidase is activated upon restoration of
transcription of the yeast galactose metabolism genes.
20

A further aspect of the present invention also
provides a method of identifying VEGF-X in a sample,
which method comprises contacting said sample with an
antibody according to the invention and monitoring for
25 any binding of any proteins to said antibody. A kit
for identifying the presence of VEGF-X in a sample is
also provided comprising an antibody according to the
invention and means for contacting said antibody with
said sample.

30

VEGF-X may be recovered and purified from recombinant
cell cultures by methods known in the art, including
ammonium sulfate or ethanol precipitation, acid
extraction, anion or cation exchange chromatography,
35 phosphocellulose chromatography, hydrophobic
interaction chromatography, affinity chromatography,
hydroxyapatite chromatography and lectin

chromatography.

5 The VEGF-X protein of the present invention may be a naturally purified product, or a product of chemical synthetic procedures, or produced by recombinant techniques from a prokaryotic or eukaryotic host (for example, by bacterial yeast, higher plant, insect and mammalian cells in culture). Depending upon the host employed in a recombinant production procedure, the
10 polypeptides of the present invention may be glycosylated with mammalian or other eukaryotic carbohydrates or may be non-glycosylated.

15 VEGF-X is particularly advantageous as a wound healing agent, where, for example, it is necessary to re-vascularize damaged tissues, or where new capillary angiogenesis is important. Accordingly, VEGF-X may be used for treatment of various types of wounds such as for example, dermal ulcers, including pressure sores, venous ulcers, and diabetic ulcers. In addition, it
20 can be used in the treatment of full-thickness burns and injuries where angiogenesis is desired to prepare the burn in injured sites for a skin graft and flap. In this case, VEGF-X or the nucleic acid encoding it
25 may be applied directly to the wound. VEGF-X may be used in plastic surgery when reconstruction is required following a burn, other trauma, or even for cosmetic purposes.

30 An important application of VEGF-X is to induce the growth of damaged bone, periodontium or ligament tissue. For example, it may be used in periodontal disease where VEGF-X is applied to the roots of the diseased teeth, leading to the formation of new bone
35 and cementum with collagen fibre ingrowths. It can be used for regenerating supporting tissues of teeth, including alveolar bone, cementum and periodontal

ligament, that have been damaged by disease and trauma.

5 Since angiogenesis is important in keeping wounds clean and non-infected, VEGF-X may be used in association with surgery and following the repair of cuts. It should be particularly useful in the treatment of abdominal wounds where there is a high risk of infection.

10 VEGF-X can also be used for the promotion of endothelialization in vascular graft surgery. In the case of vascular grafts using either transplanted or synthetic material, VEGF-X may be applied to the
15 surface of the graft or at the junction to promote the growth of the vascular endothelial cells. One derivation of this is that VEGF-X can be used to repair the damage of myocardial and other occasions where coronary bypass surgery is needed by stimulating
20 the growth of the transplanted tissue. Related to this is the use of VEGFX to repair the cardiac vascular system after ischemia.

25 The protein of the present invention may also be employed in accordance with the present invention by expression of such protein *in vivo*, which is often referred to as "gene therapy".

30 Thus, for example, cells such as bone marrow cells may be engineered with a polynucleotide (DNA or RNA) encoding for the protein *ex vivo* as defined herein, the engineered cells are then provided to a patient to be treated with the polypeptide. Such methods are well-known in the art. For example, cells may be
35 engineered by procedures known in the art by use of a retroviral particle containing RNA encoding for the protein of the present invention.

Similarly, cells may be engineered *in vivo* for expression of the protein *in vivo*, for example, by procedures known in the art.

- 5 A further aspect of the invention comprises a method of treating a disorder mediated by expression of a protein according to the invention, by administering to a patient an amount of an antisense molecule as described herein, in sufficient concentration to
10 alleviate or reduce the symptoms of said disorder.

- Compounds which inhibit or enhance angiogenesis may be identified by providing a host cell or organism according to the invention or a transgenic cell,
15 tissue or organism according to the invention, contacting a test compound with said cell, tissue or organism and monitoring for the effect of said compound compared to a cell tissue or organism which has not been contacted with said compound. These
20 compounds may themselves be used as a medicament or included in a pharmaceutical composition for treatment of disorders mediated by inappropriate vascularisation or angiogenic activity.

- 25 The present inventors have also, advantageously, identified in the sequence encoding the VEGF-X protein a CUB domain, which has heretofore not previously been identified in VEGF-type growth factors. The VEGF-X protein may therefore exert dual regulatory effects
30 via interaction with the VEGF tyrosine kinase receptors or with neuropilin receptors mediated by the CUB domain. Thus, the sequence encoding said CUB domain may be included in an expression vector for subsequent transformation of a host cell, tissue or
35 organism.

VEGF-X or fragments thereof may be able to modulate

the effects of pro-angiogenic growth factors such as VEGF as indicated in the findings presented in the examples below that the N-terminal part of the VEGF-X protein, a CUB-like domain, is able to inhibit VEGF-stimulated proliferation of HUVECs. VEGF-X or fragments thereof may therefore be useful in therapy of conditions involving inappropriate angiogenesis. Inhibition of the angiogenic activity of VEGF has been linked with inhibition of tumour growth in several models eg Kim K. J. et al, Nature 362:841-844, (1993). Additionally, agents able to inhibit angiogenesis would be expected to be useful in treating other angiogenesis-dependent diseases such a retinopathy, osteoarthritis and psoriasis (Folkman, J., Nature Medicine 1:27-31, (1995).

As identified in more detail in the Examples described herein the present inventors have surprisingly identified that the CUB domain of VEGF-X is able to inhibit stimulation of proliferation of HUVECs induced by either VEGF or bFGF. The CUB domain may, therefore, be utilised as a therapeutic agent for inhibition of angiogenesis and for treatment of condition associated with inappropriate vascularisation or angiogenesis.

Therefore according to a further aspect of the invention there is provided a method of inhibiting angiogenic activity and inappropriate vascularisation including formation and proliferation of new blood vessels, growth and development of tissues, tissue regeneration and organ and tissue repair in a subject said method comprising administering to said subject an amount of a polypeptide having an amino acid sequence from position 40 to 150 of the sequence illustrated in Figure 10 or a nucleic acid molecule encoding the CUB domain according to the invention in

sufficient concentration to reduce or prevent said angiogenic activity.

5 Furthermore there is also provided a method of
treating or preventing any of cancer, rheumatoid
arthritis, psoriasis and diabetic retinopathy, said
method comprising administering to said subject an
amount of a polypeptide having an amino acid sequence
10 from position 40 to 150 of the sequence illustrated
in Figure 10 or a nucleic acid molecule encoding the
CUB domain according to the invention in sufficient
concentration to treat or prevent said disorders.

15 The CUB domain may also be used to identify compounds
that inhibit or enhance angiogenic activity such as
inappropriate vascularisation, in a method comprising
contacting a cell expressing a VEGF receptor and/or a
neuropilin 1 or 2 type receptor with said compound in
the presence of a VEGF-X protein according to the
20 invention and monitoring for the effect of said
compound or said cell when compared to a cell which
has not been contacted with said compound. Such
compounds may then be used as appropriate to prevent
or inhibit angiogenic activity to treat the disorders
25 or conditions described herein, or in a
pharmaceutical composition. An antibody to said CUB
domain may also be useful in identifying other
proteins having said sequences.

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Deposited Plasmids

		<u>Date of Deposit</u>	<u>Accession No.</u>
	Plasmid VEGFX/pCR2.1		
5	1TOPO FL	1 March 1999	LMBP 3925
	Plasmid VEGFX/prSETB BD		
	amino acids	1 March 1999	LMBP 3926
10	G230-G345		
	Plasmid VEGFX/pcR.2.1		
	FL Clone 9	20 October 1999	LMBP 3977
15	Plasmid VEGF-X CUB		
	PET22b	20 December 1999	-----
20	The above plasmids were deposited at the Belgian Coordinated Collections of Microorganisms (BCCM) at Laboratorium Voor Moleculaire Biologie-Plasmidencollectie (LMBP) B-9000, Ghent, Belgium, in accordance with the provisions of the Budapest Treaty of 28 April 1977.		
25	The invention may be more clearly understood with reference to the accompanying example, which is purely exemplary, with reference to the accompanying drawings, wherein:		
30	Figure 1:	is a DNA sequence identified in the Incyte LifeSeq™ database coding for a novel VEGF-X protein.	
35	Figure 2:	is an illustration of amino acid sequence of the nucleic acid sequence of Figure 1.	

Figure 3: is an illustration of PCR primer sequences utilised to identify the VEGF-X protein according to the invention.

5

Figure 4: is a diagrammatic illustration of the spatial relationships in the VEGF-X sequence of the clones identified using the PCR primer sequences of Figure 3.

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Figure 5: is an illustration of the nucleotide sequences of the 5' RACE primers used to identify the 5' end of the VEGF-X open reading frame.

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Figure 6: is an illustration of the sequence obtained from the RACE experiment.

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Figure 7: is an illustration of the nucleotide sequences obtained from the search of LifeSeq™ database using the sequence in Figure 6.

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Figure 8: is an illustration of the primers used to clone the entire coding sequence of VEGF-X.

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Figure 9: is an illustration of the entire coding sequence of VEGF-X.

Figure 10: is an illustration of the predicted amino acid sequence of the nucleotide sequence of Figure 9.

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Figure 11: is an alignment of the sequence of

Figure 10 with the sequences of VEGF-A to D.

- 5 Figure 12: is an illustration of variant sequences of the VEGF-X protein according to the invention.
- 10 Figure 13: is an illustration of the oligonucleotide primers used for E.coli expression of VEGF-X domains and for expression of the full length sequence of VEGF-X in a baculovirus/insect cell expression system.
- 15 Figure 14: depicts nucleic acid sequences of 18 human EST clones obtained from a BLAST search of the LifeSeq™ database used to identify the full sequence encoding VEGF-X.
- 20 Figure 15: depicts the nucleotide sequences of 50 human EST clones obtained from the LifeSeq™ database.
- 25 Figure 16: is an illustration of nucleotide sequences utilised as primers to identify the nucleotide sequence encoding VEGF-X.
- 30 Figure 17: is a nucleotide sequence coding for a partial VEGF-X protein according to the invention.
- 35 Figure 18: is an illustration of a partial nucleotide sequence encoding VEGF-X protein according to the invention.

5 Figure 19: is an illustration of a DNA and polypeptide sequence used for mammalian cell expression of VEGF-X. The predicted VEGF-X signal sequence is in lower case letters. The C-terminal V5 epitope and His6 sequences are underlined.

10 Figure 20: is an illustration of a DNA and polypeptide sequence used for baculovirus/insect cell expression of VEGF-X. In the polypeptide sequence the signal sequence is shown in lower case. The N-terminal peptide tag added to the predicted mature VEGF-X sequence is underlined.

20 Figure 21: is an illustration of a DNA and polypeptide sequence used for *E. coli* expression of VEGF-X. The polypeptide sequences at the N- and C- termini derived from the MBP fusion and His6 tag respectively are underlined.

25 Figure 22: illustrates the disulphide-linked dimerisation of VEGF-X. Protein samples were analysed by SDS-PAGE. Prior to loading the gel, samples were heated to 95°C for 5 minutes in sample buffer in the presence (+) or absence (-) of reducing agent. (A) samples from COS cell expression of a C-terminally V5/His6 peptide-tagged construct. The left hand panel is total conditioned medium, the right hand panel is material purified on Nickel agarose resin. Reduced monomer

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5 and putative disulphide-linked, non-reduced dimer are indicated by arrows. There appears to be proteolysis of the protein during purification. Gels were
10 blotted onto nylon membranes and protein detected with an anti V5 monoclonal antibody. (B) Samples from *E.coli* expression of a maltose-binding protein/His6 dual fusion construct. M indicates the molecular weight markers (Benchmark, LifeTechnologies). The gel was stained with Coomassie Blue by standard procedures. The fusion protein has an apparent molecular
15 weight of 80kDa.

Figure 23: illustrates the glycosylation of VEGF-X. VEGF-X was purified from the culture supernatant of COS cells transfected with the pcDNA6/V5-His construct. Supernatants were harvested 72h post-transfection and purified on nickel resin. Samples were then treated with EndoH (+) or untreated (-) before SDS-PAGE and blotting, as described in the legend to Figure 22.
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Figure 24: is an illustration of the DNA and polypeptide sequence used for *E. coli* expression of the VEGF-like domain of VEGF-X. Polypeptide sequences at the N-terminus of the protein derived from the vector are underlined.
30

35 Figure 25: shows expression of the VEGF-X VEGF domain in *E. coli*. Lane 1-10µl broad

range marker (New England Biolabs),
lane 2-10µl unreduced sample, lane 3-
10µl reduced sample. The reduced PDGF
domain protein (lane 3) has an
apparent molecular weight of
approximately 19kDa on SDS-PAGE.

5

Figure 26: illustrates a DNA and polypeptide
sequence used for *E. coli* expression
of the CUB-like domain of VEGF-X. The
polypeptide sequence at the N-terminus
derived from the vector-encoded signal
and the introduced His6 tag are
underlined.

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Figure 27: shows expression of the VEGF-X CUB
domain in *E. coli*. The CUB domain
protein was purified on Nickel chelate
resin. The protein migrates at
approximately 23kDa on SDS-PAGE.

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Figure 28: illustrates the effect of truncated
VEGF-X (CUB domain) on HUVEC
proliferation. (A) Human Umbilical
Vein Endothelial Cells (one-day-
treatment). (B) Human Umbilical Vein
Endothelial Cells (24-hour starving
followed by one-day-treatment). (C)
Effect of VEGF-A₁₆₅ and VEGF-X CUB
domain on the proliferation of HUVEC
(two-day-treatment).

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Figure 29: depicts the tissue distribution of
VEGF-X mRNA analysed by Northern
blotting and RT-PCR in (A) normal
tissues and (B) tumour tissue and cell
lines.

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- Figure 30: depicts the partial intron/exon structure of the VEGF-X gene. (A) Genomic DNA sequences of 2 exons determined by sequencing; exon sequence is in upper case, intron sequence is in lower case. (B) Shows the location of splice sites within the VEGF-X cDNA sequence. The location of mRNA splicing events is indicated by vertical lines. The cryptic splice donor/acceptor site at nt. 998/999 (diagonal lines) gives rise to the splice variant forms of VEGF-X. No splice site information is given for the region shown in italics.
- Figure 31: is a graphic representation of the effect of FL-VEGF-X on HuVEC proliferation: (24 hour serum starvation followed by one day treatment).
- Figure 32: is a graphic representation of the combined effect of truncated VEGF-X (CUB domain) and human recombinant VEGF₁₆₅ on HuVEC proliferation: (24 hour serum starvation followed by two day treatment).
- Figure 33: is a graphic representation of the combined effect of the CUB domain and human recombinant bFGF on HuVEC proliferation: (24 hour serum starvation followed by two day treatment).
- Figure 34: is a graphic representation of the

results of a LDH assay for testing cytotoxicity of the CUB domain or the CUB domain with rhVEGF₁₆₅.

5 Figure 35: is a graphic representation of the results obtained from a LDH assay for testing cytotoxicity of the CUB domain or CUB domain with rh-bFGF.

10 A BLAST (Basic Local Alignment Search Tool; Altschul et al., 1990 J. Mol. Biol. 215, 403-410) search was performed in the proprietary LifeSeq™ human EST database (Incyte Pharmaceuticals, Inc., Palo Alto, CA, USA). BLAST produces alignments of both
15 nucleotide and amino acid sequences to determine sequence similarity. Because of the local nature of the alignments, BLAST is especially useful in determining exact matches or in identifying homologues. While it is useful for matches which do
20 not contain gaps, it is inappropriate for performing motif-style searching. The fundamental unit of BLAST algorithm output is the High-scoring Segment Pair (HSP).

25 Eighteen human EST clones (Figure 14) with high similarity to the previously identified VEGF proteins were identified and a further fifty EST clones (Figure 15) were identified using these sequences as query sequences, allowing us to deduce the putative
30 sequence for the new VEGF-X protein. The sequences obtained were compared to known sequences to determine regions of homology and to identify the sequence as a novel VEGF-type protein. Using the DNA sequence information in the databases we were able to
35 prepare suitable primers having the sequences of VEGF-X 1-10 illustrated in Figure 3 for use in subsequent RACE experiments to obtain the complete

DNA sequence for the VEGF-X gene.

Cloning

5 A profile was developed based on the VEGF-like domain,
in existing VEGF sequences (VEGF-A, B, C and D).
This was used to search the public databases and the
Incyte LifeSeq™ database. No significant novel
10 matching sequences were found in the public
databases. All of the matching sequences found in
the LifeSeq™ database (~1000) were assembled to give
a smaller number of sequences (~30), which included
the known VEGFs and a potential novel VEGF (figures
1 and 2). This sequence was named VEGF-X.
15
Oligonucleotides were designed to amplify the VEGF-X
sequence from cDNA (figure 3). The ESTs found in
LifeSeq™ were from a range of tissues, with a slight
predominance of sequences from ovary, testis,
20 placenta and lung (Figure 14 and 15). Accordingly
the oligonucleotides were used to amplify cDNA
derived from lung and placenta. First-round PCR
products were found at ~200bp larger than the
expected sizes, while 3 major species appeared after
25 a second round of PCR amplification, the smallest of
which was of the expected size. These fragments were
cloned and sequenced. The smallest fragment did
indeed have the sequence originally identified from
the LifeSeq database, while the others contained
30 insertions (figure 4).

As the first round of amplification suggested that
the major species found in cDNA from ovary and
placenta was not that originally identified in the
35 LifeSeq™ database, the focus of effort was switched
to the presumed major species (it seemed likely that

clones 57, 25-27 and 2.1kb clones 1-3 in fig 4 represented the major mRNA species). Conceptual translation of the DNA sequences of these cloned PCR fragments indicated that the complete open reading
5 frame was not present in the clones or in the sequence from LifeSeq™. While all clones contained the same sequence in the region of the translation termination codon, indicating that the end of the open reading frame had been identified, the 5' end of
10 the open reading frame had not been cloned. 5' RACE experiments were therefore carried out in order to find the start of the reading frame. PCR primers designed for RACE experiments are shown in figure 5. RACE PCR products were sequenced directly. Sequence
15 could be obtained from the 3' end of these RACE products but not from the 5' end; probably because the products were not cloned and were therefore heterogeneous at the 5' end. This new sequence was assembled with the existing cloned sequence to give
20 the sequence shown in figure 6. Searching the LifeSeq™ database with this sequence identifies ESTs which extend the sequence a further 140bp in the 5' direction and a further 160bp in the 3' direction (figure 7). This longer contig was used to design
25 oligonucleotide primers to amplify the entire coding sequence (these primer sequences are shown in figure 8). PCR was carried out using primers 5'-1 and vegfX10 (in order to clone a "full-length" cDNA), and with primers 5'-1 and vegfX6 (in order to clone the
30 full coding region, see figure 3 for sequences of vegfX10 and vegfX6). A number of clones were obtained for the shorter fragment, of which clones 4 and 7 contain no PCR errors (sequence of clones 4 & 7 in figure 9). A single clone was obtained for the
35 longer fragment (clone 9), but this sequence appears to contain 2 PCR errors.

The predicted polypeptide from these longer contigs is shown in figure 10. Amino acids 1-22 are predicted to encode a signal sequence (von Heijne, 1986, *Nucleic Acids Res.* 14, 4683-4690). Figure 11 shows an alignment of the protein sequence with VEGFs A-D. The region homologous to the other VEGFs is located towards the C-terminus of the protein. As the VEGF homology domain is expected to belong to the TGF-beta superfamily of growth factors and to consist of a dimer containing both intra- and intermolecular disulphide bonds, initial alignments focussed on the cysteines. However, mapping of the sequence onto the known x-ray structure of the VEGF-A receptor-binding domain (Muller et al (1997) *Proc. Natl. Acad. Sci USA* 94, 7192-7197) suggests that the alignment in figure 11 is plausible, as the extra 4 cysteine residues within the VEGF-homology region of VEGF-X (compared to this region of VEGF-A) correspond to residues which are spatially close in VEGF-A, and may therefore be able to form disulphide bonds.

A search of the PFAM database of protein domains with the full-length polypeptide sequence from figure 10 identifies two domain consensus sequences within the polypeptide. The more C-terminal domain is a "VEGF" domain: (the known VEGFs all contain this domain and the structure of this region of VEGF-A is similar to that of PDGF). Additionally towards the N-terminus of the polypeptide there is a CUB domain (amino acids ~40-150). The CUB domain is a 100-110 amino acid extracellular domain found in a number of developmentally-regulated proteins. When the full-length protein is used to search the protein databases using the BLAST 2 algorithm, the scores for matches to CUB domain-containing proteins are more

significant than those to the other VEGFs.

Interestingly, the most significant matches are to the CUB domains of Neuropilins, and Neuropilin-1 was recently identified as a receptor of one of the VEGF-A isoforms VEGF-A₁₆₅ (Soker et al. (1998) Cell 92, 735-745).

Assuming that the variant sequences isolated by PCR (i.e. the smaller PCR fragments) use the same translation initiation site as the full-length sequence, they would result in production of the variant proteins shown in figure 12. It may be significant that both of these variant proteins retain the CUB domain and delete all or part of the VEGF-like domain. The production of these variant sequences can be explained by the use of a cryptic splice donor/acceptor site within the VEGF-X sequence (figure 30B, between nt. 998/999): one variant arises by splicing out of the region between nt. 729-998, the other by splicing out of the region between nt. 999-1187.

Expression

25 Full-length expression constructs

Mammalian cells

Clone 4 containing the full CDS of VEGF-X (see figure 9), was used to generate constructs for expression of full-length protein. The sequence was amplified by PCR and cloned into the vector pCDNA6/V5-His so as to add a C-terminal V5 epitope tag and His₆ tag. The DNA and polypeptide sequence in this vector is shown in figure 19. Transient expression in COS cells followed by western blotting and detection via an anti-V5 mAb demonstrates the secretion of a protein of ~50K into the medium in transfected cells only

(figure 22A). This construct can also be used to generate VEGF-X expressing stable CHO cell lines.

Baculovirus/Insect-cell expression system

5 For expression in the baculovirus/insect cell system the DNA encoding the predicted mature VEGF-X polypeptide sequence was fused to a sequence encoding a signal derived from melittin, a secreted insect protein. An N-terminal 6His tag was also added to
10 facilitate purification. The insert was then cloned into the baculovirus expression vector pFASTBAC. The DNA and polypeptide sequence of this construct is shown in figure 20. Infection of *Trichoplusia ni* Hi5 cells with this recombinant baculovirus results in
15 the secretion of a protein of approximately 45K into the medium (data not shown).

E.coli

20 The coding region of VEGF-X has been cloned in a variety of ways for expression as a secreted protein in *E.coli*. A particularly useful expression clone carries an N-terminal fusion to the *E.coli* maltose-binding protein (MBP- derived from the expression vector pMAL-p2, New England Biolabs) and a
25 C-terminal fusion to a 6His tag. The DNA and polypeptide sequence of this vector is shown in figure 21. Sequential purification of cell fractions on Ni-NTA resin and amylose resin allows the isolation of the expressed protein (see figure 22B).

30

Expression of fragments

VEGF

The VEGF domain of VEGF-X has been expressed in *E.coli*. Similar domains from VEGF-A (Christinger et
35 al. (1996) *PROTEINS: Structure, Function and Genetics* 26, 353-357), and VEGF-D (Achen et al (1998) *Proc.*

Natl. Acad. Sci USA 95, 548-553) have been shown to be capable of binding to the respective receptors. Expression of these domains was carried out using the bacterium *E.coli*. Additionally, the full-length protein was expressed using the baculovirus/insect cell expression system. The oligonucleotide primers which have been obtained for these experiments are shown in figure 13. The construct directed expression in the bacterial cytoplasm, and as expected the protein was produced in insoluble form in inclusion bodies (the DNA and polypeptide sequence used for PDGF domain expression is shown in figure 24). Inclusion bodies were washed, solubilized with urea and the protein purified under denaturing conditions, before refolding by dialysis to remove the urea. Soluble protein was obtained, but shows little evidence of the disulphide bond linked dimers seen with material derived from animal cells (figure 25, compare with figure 22A & B). It is not clear therefore whether this protein is correctly folded.

CUB

The CUB domain has been expressed as a soluble secreted protein in *E.coli* (figure 26). The protein was purified by binding to Ni-NTA resin (figure 27) and assayed for activity on HUVECs in an in-vitro proliferation assay.

Properties of the VEGF-X protein

The transient mammalian cell expression system described above has been used to generate full-length VEGF-X protein, as shown by antibody detection following Western blotting (see figure 22A).

Disulphide bond linked dimers

The other members of the PDGF family of growth

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cleavage between the CUB and VEGF domains. EndoH treatment of the preparation gives a slight mobility change for the full-length protein (figure 23), but for the smaller VEGF domain fragment there is a clear change, indicating that the predicted glycosylation site within the VEGF domain at residue 254 is indeed glycosylated.

Activity of proteins in cell-based assays

Protein samples were tested for activity in cell proliferation, cell migration and *in-vitro* angiogenesis assays. Active samples can also be tested in the *in vivo* matrigel mouse model of angiogenesis.

Full-length VEGF-X protein

Conditioned medium derived from COS cells transiently expressing VEGF-X (see figure 22A) displayed no detectable activity in any of the assays. However, as VEGF-X protein could only be detected in this preparation by Western blotting, and not by Coomassie-staining of gels, it is clearly present at very low levels and this may be the reason for the observed lack of activity in the cell proliferation, migration or *in vitro* angiogenesis tests.

VEGF domain

The VEGF domain protein described above has been tested in cell proliferation (on a range of cell types), cell migration and *in vitro* angiogenesis assays and has failed to show activity in any of these tests. As suggested above, this may be due to incorrect folding of this protein.

CUB domain

The CUB domain protein at the highest dose tested

(1pg/ml) appears to inhibit proliferation of HUVECs in the absence of other stimulation (figure 28A & B). This effect is also seen following stimulation with the lowest VEGF-A₁₆₅ dose tested (1ng/ml- figure 28C).

- 5 The CUB domain of VEGF-X therefore appears to show antiproliferative activity on HUVECs, even in the presence of low VEGF-A₁₆₅ doses.

Tissue distribution of mRNA

- 10 VEGF-A mRNA expression has been shown to be upregulated in a wide variety of human tumors (lung, breast, ovarian, colon, stomach, liver, pancreas, kidney, bladder and prostate- Takahashi et al, 1995). Tumor VEGF-A expression has been shown to correlate
15 with tumor growth rate, microvascular density and tumor metastasis (Takahashi et al, 1995). It was thus of interest to examine the mRNA expression patterns of VEGF-X. Accordingly, Northern blot analysis of mRNA derived from different tissues has
20 been carried out. The results indicate that although the VEGF-X mRNA is expressed at low levels, it is present in a wide range of tissues. PCR amplification of cDNA from a range of tissue sources supports this idea (figure 29A). The major mRNA
25 species is approximately 3.1kb in size. There is no significant upregulation seen in tumour cell lines or in tumour tissues tested (figure 29B), with the possible exception of the cell lines GI-117 (lung carcinoma) and SaOS-2 (osteosarcoma). The results of
30 these initial tissue distribution studies do not, therefore, provide evidence for upregulation of VEGF-X in tumour growth, as is seen with VEGF-A.

Genomic structure of the VEGF-X gene

- 35 A genomic BAC clone covering the 3' part of the VEGF-X locus was isolated by hybridisation screening

of nylon filters containing a human BAC library. Direct sequencing of this clone using oligonucleotide primers based on the VEGF-X cDNA sequence allowed the determination of several intron/exon boundaries (figure 30). Interestingly, the position of the mRNA splice site within the PDGF domain (nt 1187/1188 in figure 30B) is conserved with respect to those in the VEGF-A and VEGF-D genes (Tischer et al, 1991; Rocchigiani et al, 1998).

Materials & Methods

PCR, Cloning, DNA sequence determination and BAC screening.

All primers were purchased from Eurogentec, Seraing, Belgium. Insert-specific sequencing primers (15- and 16-mers) were designed by visual inspection of the DNA sequences. DNA was prepared on Qiagen-tip-20 columns or on Qiaquick spin columns (Qiagen GmbH, Düsseldorf, Germany) and recovered from the spin columns in 30µl Tris/EDTA-buffer (10mM TrisHCl pH 7.5, 1 mM EDTA (sodium salt)). Sequencing reactions were performed using BigDye™ Terminator Cycle Sequencing Ready Reaction kits (Perkin Elmer, ABI Division, Foster City, CA, USA) and were run on an Applied Biosystems 377 DNA sequencer (Perkin Elmer, ABI Division, Foster City, CA, USA). Polymerase chain reactions were carried out according to standard procedures (Ausubel et al, 1997). The PCR fragments were cloned into vectors pCR2.1 (Invitrogen, Carlsbad, CA, USA) or pCR-TOPO (Invitrogen, NL) according to the manufacturer's instructions. One of those vectors, plasmid VEGFX/pCR2.1 1TOPO FL was deposited on 1 March 1999 under Accession No. LMBP 3925. After sequence determination, the inserts were cloned into the desired expression vectors (see

figures 19, 20, 21, 24 & 26).

5 A human genomic BAC library (Genome Systems, Inc., St
Louis, MI, USA) was screened by hybridisation to
oligonucleotides derived from the VEGF-X cDNA
sequence, according to the manufacturer's
instructions. BAC DNA was prepared using a Qiagen
plasmid midi kit (Qiagen GmbH, Düsseldorf, Germany)
10 according to the manufacturer's instructions with
some modifications (after clearing of the lysate from
chromosomal DNA, supernatants from individual
preparations were pooled on a single column (tip
100), and after the 70 % EtOH wash, the pellet was
resuspended overnight at 4°C in 100 µl TE). 20-mer
15 sequencing primers were designed based on the known
cDNA sequence, and sequencing carried out as above.

5' RACE

20 In order to extend the cDNA clone in a 5' direction
RACE reactions were carried out. Since it was known
that the mRNA is present in placenta and skeletal
muscle, Marathon-Ready™ placenta and skeletal muscle
cDNAs were purchased from Clontech (Palo Alto CA.
25 USA) and used according to the manufacturer's
instructions. DNA fragments were excised from
agarose gels, purified using QiaQuick PCR
purification columns (Qiagen GmbH, Düsseldorf,
Germany) and sequenced directly.

30

VEGF-X protein expression and purification

DNA fragments encoding the desired protein sequences
were amplified by PCR and cloned into appropriate
expression vector systems.

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For mammalian cell expression, the full coding

sequence was cloned into the vector pcDNA6/V5-his (Invitrogen Leek, NL, see figure 19 for construct sequence), so as to add a C-terminal peptide tag to assist in detection and purification.

5

For insect cell expression the sequence of the predicted mature polypeptide was initially amplified to add an N-terminal 6His peptide and then cloned into the pMelBacB vector (Invitrogen, Leek, NL) to add an insect cell signal sequence. The entire insert was then PCR-cloned into the vector pFASTBAC-1 (LifeTechnologies, Gaithersburg, MA, USA) for construction of a baculovirus according to the manufacturer's instructions.

15

For *E.coli* expression, the coding region was PCR amplified to add a C-terminal 6His tag and then cloned into the vector pMAL-p2 (New England Biolabs, Beverly, MA, USA). The coding sequence of this construct is shown in figure 21). The protein was purified first on Ni-NTA resin (Qiagen GmbH, Düsseldorf, Germany) and then on amylose resin (New England Biolabs, Beverly, MA, USA), according to the manufacturers' instructions.

25

DNA sequences encoding the CUB and VEGF domain fragments of VEGF-X were PCR amplified and cloned into pET22b and pET21a (Novagen, Madison, WI, USA) respectively. The CUB domain protein was prepared either from the periplasm or medium of induced cultures by standard methods (Ausubel et al, 1997). The protein was initially purified by precipitation with 20% ammonium sulphate. After overnight dialysis vs 20mM Tris Hcl pH7.5, 100mM NaCl to remove ammonium sulphate, the protein was further purified on Ni-NTA resin as described above. The VEGF domain protein was expressed in insoluble form, and preparation of

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inclusion bodies was carried out using standard procedures (Ausubel et al 1997). Inclusion bodies were dissolved in 6M guanidine hydrochloride, 20mM Tris Hcl pH8.0, 200mM NaCl, 1mM 2-mercaptoethanol, and purified on Ni-NTA resin (Qiagen GmbH, Düsseldorf, Germany) according to the manufacturer's instructions. The protein was refolded by dialysis against several changes of buffer containing decreasing concentrations of denaturant.

Analysis of protein glycosylation was carried out using EndoH (Roche Molecular Biochemicals, Brussels, BE) according to the manufacturer's instructions.

Cell Proliferation Assay

Human umbilical vein endothelial cells (HUVECs) (Clonetics, San Diego, CA.) were trypsinized with 0.05% trypsin/0.53mM EDTA (Gibco, Gaithersburg, MD.), resuspended in the EGM-2 (Clonetics, San Diego, CA.), counted, and distributed in a 96-well tissue culture plate at 5,000 cells/well. Following cell attachment and monolayer formation (16 hours), cells were stimulated with various concentrations of truncated VEGF-X (CUB domain or VEGF domain) or dilutions of culture supernatants of the full-length VEGF-X (COS 7 or HEK293) in DMEM (Gibco, Gaithersburg, MD.) containing 0.5% to 2% FBS (HyClone, Logan, UT) as indicated. For human fetal dermal fibroblasts (American Type Culture Collection, Rockville, MD.), the growth medium was replaced by DMEM containing 0.1% BSA (Sigma, St. Louise, MO.) with or without various concentrations of truncated VEGF-X proteins. For HCASMC (Clonetics, San Diego, CA.), the medium was replaced by DMEM containing 0.5% FBS. The cells were treated for a further 24 hr-72 hr. For the measurement of proliferation, the culture media were replaced with 100 µl of DMEM containing 5% FBS and 3

5 μ Ci/ml of [3H]-thymidine (Amersham, Arlington Heights, IL.). Following pulse labeling, cells were fixed with methanol/acetic acid (3:1, vol/vol) for 1 hour at room temperature. The cells were washed twice with 250 μ l/well of 80% methanol. The cells were solubilized in 0.05% trypsin (100 μ l/well) for 30 minutes then in 0.5% SDS (100 μ l/well) for another 30 minutes. Aliquots of cell lysates (180 μ l) were combined with 2 ml of scintillation cocktail (Fisher, Springfiled, NJ) and the radioactivity of cell lysates was measured using a liquid scintillation counter (Wallac 1409). In each case, samples were performed in quadruplicate.

15 **Chemotaxis Assay**

20 The chemotactic response of HUVECs was assayed using a 48-well modified Boyden chamber (NeuroProbe, Cabin John, MD.) and collagen-coated (0.1mg/ml type I collagen, Collaborative Biomedical, Bedford, MA.) polycarbonate membrane filters with a pore diameter of 8 μ m (NeuroProbe, Cabin John, MD.). Cell suspensions (15,000/well) were loaded to the upper part of the chemotaxis chamber and stimulated for 4 hours with rhVEGF₁₆₅ (0.1-10 ng/ml) (Calbiochem, San Diego, CA.) or various concentrations of truncated VEGF-X (PDGF domain). Cells remaining on the top of the membrane were removed. Migration was assessed by counting the number of cells that migrated to the lower side of the filter membrane. The membrane was fixed with 10% formaldehyde for 15 min, followed by staining with Gill's hemotoxylin III (Poly Scientific, Bay Shore, NY.). The assay was performed in triplicates and six independent high power fields per well were counted using a light microscope at 250 magnification. The results were expressed as the fold of unstimulated cells (EGM containing 0.1% BSA).

In Vitro Angiogenesis Assay

In vitro angiogenesis in fibrin gels was quantitated using spheroids of human umbilical vein endothelial cells (Korff et al., 1998). To generate endothelial cell spheroids of defined size and cell number, a specific number of cells (~ 800 cells per spheroid) was suspended in EGM-2 culture medium containing 20% methylcellulose (Sigma, St. Louis, MO.), seeded into nonadherent round-bottom 96-well plates. All suspended cells in one well contributed to the formation of a single endothelial cell spheroid within 24 hours. A fibrin gel stock solution was prepared freshly prior to use by mixing 3mg/ml fibrinogen (Calbiochem, San Diego, CA.) in Medium 199 (Gibco, Gaithersburg, MD.). Assays were performed in 24-well culture plates. The 1ml fibrinogen stock was mixed with 50 HUVEC spheroids and the corresponding test substance including rh-VEGF₁₆₅ or various concentration of VEGF-X. The spheroid-containing fibrinogen was rapidly transferred into 24-well plates. Fifteen microliters of thrombin (100 NIH U/ml stock, Sigma, St. Louis, MO.) was added to the gel for the fibrin gel formation. The gel formation usually occurred within 30 seconds. After gel formation, 1ml/well of Medium 199 supplemented with 20% FBS, 1mg/ml ϵ -aminocaproic acid (Calbiochem, San Diego, CA.) and antibiotics were added. The gel was incubated at 37°C (5%CO₂, 95% air, 100% humidity). After 3 days, *in vitro* angiogenesis was quantitated by measuring the length of the three longest capillary sprouts that had grown out of each spheroid (100X magnification), analyzing at least 10 spheroids per experimental group and experiment.

35

Matrigel Mouse Assay

The matrigel mouse assay is carried out as described by Passanti et al (1992).

Analysis of VEGF-X gene expression by RT-PCR analysis.

5 Oligonucleotide primers VEGF-E2 and VEGF-X14 (figure 16; figure 5) were used for the specific PCR amplification of a 350 bp fragment from VEGF-X. PCR amplifications were performed on human multiple
10 tissue cDNA (MTC™) panels (Clontech human MTC panels I and II and human Tumor MTC panel) normalised to the mRNA expression levels of six different housekeeping genes. In addition, cDNA was made from different tumor cell cultures (Caco-2 colorectal
15 adenocarcinoma; T-84 colorectal carcinoma; MCF-7 breast adenocarcinoma; T-47D breast ductal gland carcinoma; HT1080 bone fibrosarcoma; SaOS-2 osteosarcoma; SK-N-MC neuroblastoma; HepG2 hepatoblastoma; JURKAT T-cell leukemia and THP-1
20 myelomonocytic leukemia). For the preparation of tumor cell cDNA, cells were homogenised and total RNA prepared using the RNeasy Mini kit (Qiagen GmbH, Hilden, Germany) according to manufacturer's instructions. 1 µg of total RNA was reverse
25 transcribed using oligo(dT)15 as a primer and 50 U of Expand™ Reverse Transcriptase (Boehringer Mannheim, Mannheim, Germany) according to the manufacturer's instructions. PCR reactions with VEGF-X-specific or glyceraldehyde-3-phosphate dehydrogenase
30 (G3PDH)-specific primers were then performed on 1 µl of this cDNA. For all cDNAs, PCR reactions with VEGF-X specific primers were performed in a total volume of 50 µl, containing 5 µl (\pm 1 ng) of cDNA, 1x Advantage KlenTaq PCR reaction buffer, 0.2 mM dNTP, 250 nM of primers VEGF-E2 and VEGF-X14 and 1 µl of
35 Advantage KlenTaq polymerase mix. Samples were heated

to 95°C for 30 s and cycling was done for 30 s at 95°C and 30 s at 68°C for 25, 30 or 35 cycles. Control reactions using specific primers that amplify a 1 kb fragment of the housekeeping gene G3PDH were also performed according to the manufacturer's instructions.

Northern blot analysis of VEGF-X.

Northern blots containing 2 µg of poly(A)-rich RNA derived from different human tissues (Clontech Laboratories; MTN™ blot, MTN™ blot II and Cancer Cell Line MTN™ blot) were hybridized according to the manufacturers instructions with a α-[³²P]-dCTP random-priming labelled (Multiprime labelling kit, Roche Diagnostics) 293 bp specific VEGF-X fragment (PinAI-StuI fragment including 92 bp of the 3' end coding region and 201 bp of the 3' untranslated region of VEGF-X). The blots were hybridized overnight at 68°C and final washes at high stringency were at 68°C in 0.1x SSC/0.1 % SDS. The membranes were autoradiographed for 1 to 3 days with intensifying screens.

Full length VEGF-X

The effect of full length VEGF-X on proliferation of HuVEC cells was determined by the ³H-Thymidine incorporation assay. HuVEC cells were serum starved for 24 hours prior to treatment with the full length VEGF-X at the concentration range from 100 pg/ml-10 µg/ml. There was no effect of VEGF-X at 100 pg/ml-10 ng/ml on endothelial cell proliferation. At the higher concentrations of FL-VEGF-X (100 ng/ml and 1 µg/ml) there was a marked inhibition of endothelial cell proliferation. This is probably due to the very high endotoxin level in the samples. The VEGF-X sample was purified in order to decrease the

endotoxin level and is currently tested in the cell proliferation assay.

The Summary from Testing the CUB Domain

5 The effect of CUB domain on inhibition of HuVEC proliferation either serum- (2%), rh-VEGF or bFGF-stimulated, was assessed by the ³H-Thymidine incorporation assay. Cells were serum starved followed by the treatment with the CUB domain and
10 various growth factors. Results showed that the CUB domain inhibited endothelial cell proliferation, either serum- (2%), rh-VEGF or bFGF-stimulated in a dose dependent manner with maximal inhibition at 10 µg/ml. There was approximately a 2-fold inhibition
15 of proliferation (at 10 µg/ml) of cells stimulated with VEGF and bFGF and nearly a 5-fold inhibition of cells stimulated with serum (2%). Results with the LDH assay showed that there was no cytotoxicity associated with the inhibition of cell proliferation
20 by the CUB domain.

Therefore, the N-terminus of the polypeptide from Figure 10 has been shown to possess a CUB domain. When database searches are carried out using the
25 full-length coding sequence the best matches (i.e. for a BLAST search, those with the lowest probability score) are found with the CUB domain rather than with the VEGF-like domain. The best match from searching release 37 of the SWISSPROT database (Feb 1999) is to
30 the CUB domain of a neuropilin from *Xenopus laevis*, and the matches to the CUB domains of human neuropilins 1 and 2 are also more significant than matches to the VEGFs.

35 This similarity is provocative, given the identification of neuropilin-1 and -2 as cellular receptors for the VEGF-A 165 (Stoker et al. 1998,

reviewed in Neufeld et al. 1999). It is plausible
therefore that VEGF-X could exert dual regulatory
effects: via interaction with the tyrosine kinase
VEGF-receptors mediated by the VEGF-like domain, as
5 well as via interaction with VEGF isoforms or with
the neuropilin receptors, mediated by the CUB
domain.

To the best of our understanding the latter would be
10 entirely novel, and searches on the most recent
release of the Incyte database do not reveal any
other proteins containing both CUB and VEGF-like
domains. This arrangement of domains suggests
possible positive or negative models of regulation:

15 Positive- the VEGF-like domain is able to interact
productively with the tyrosine kinase VEGF receptors
giving activation, and the CUB domain is able to
interact productively with the neuropilin receptor
20 giving activation.

Negative- the VEGF-like domain does not interact
productively with the tyrosine kinase VEGF receptors,
either preventing receptor dimerisation or blocking
25 the VEGF binding sites. Further, the CUB domain does
not interact productively with the neuropilin
receptors, either preventing receptor activation or
blocking the VEGF binding sites, or indeed by binding
to VEGF isoforms and preventing their interaction
30 with receptors.

TABLE 1

	<u>ORIGINAL RESIDUE</u>	<u>EXEMPLARY SUBSTITUTIONS</u>
	ALA	SER, THR
5	ARG	LYS
	ASN	HIS, SER
	ASP	GLU, ASN
	CYS	SER
	GLN	ASN, HIS
10	GLU	ASP, GLU
	GLY	ALA, SER
	HIS	ASN, GLN
	ILE	LEU, VAL, THR
	LEU	ILE, VAL
15	LYS	ARG, GLN, GLU, THR
	MET	LEU, ILE, VAL
	PHE	LEU, TYR
	SER	THR, ALA, ASN
	THR	SER, ALA
20	TRP	ARG, SER
	TYR	PHE
	VAL	ILE, LEU ALA
	PRO	ALA

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10

Sequence ID No 1

5

10

15

20

25

Sequence ID No 7 corresponds to the polynucleotide sequence of VEGFX4 illustrated in Figure 3.

35

Sequence ID No 9 corresponds to the polynucleotide sequence of VEGFX6 illustrated in

Figure 3.

5	Sequence ID No 10	corresponds to the polynucleotide sequence of VEGFX7 illustrated in Figure 3.
10	Sequence ID No 11	corresponds to the polynucleotide sequence of VEGFX8 illustrated in Figure 3.
15	Sequence ID No 12	corresponds to the polynucleotide sequence of VEGFX9 illustrated in Figure 3.
20	Sequence ID No 13	corresponds to the polynucleotide sequence of VEGFX10 illustrated in Figure 3.
25	Sequence ID No 14	corresponds to the polynucleotide sequence of VEGFX11 illustrated in Figure 4.
30	Sequence ID No 15	corresponds to the polynucleotide sequence of VEGFX12 illustrated in Figure 4.
35	Sequence ID No 16	corresponds to the polynucleotide sequence of VEGFX13 illustrated in Figure 4.
	Sequence ID No 17	corresponds to the polynucleotide sequence of VEGFX14 illustrated in Figure 4.
	Sequence ID No 18	corresponds to the polynucleotide sequence 5'-1 in Figure 8.

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	Sequence ID No 19	corresponds to the polynucleotide sequence 5'-2 in Figure 8.
5	Sequence ID No 20	corresponds to the polynucleotide sequence of VEGFX6 illustrated in Figure 13.
10	Sequence ID No 21	corresponds to the polynucleotide sequence of VEGFX7 illustrated in Figure 13.
15	Sequence ID No 22	corresponds to the polynucleotide sequence of VEGFX8 illustrated in Figure 13.
	Sequence ID No 23	corresponds to the polynucleotide sequence of VEGFX9 illustrated in Figure 13.
20	Sequence ID No 24	corresponds to the polynucleotide sequence of VEGBAC1 illustrated in Figure 13.
25	Sequence ID No 25	corresponds to the polynucleotide sequence of VEGBAC2 illustrated in Figure 13.
30	Sequence ID No 26	corresponds to a polypeptide having the amino acid sequence from amino acid position 40 to 150 of the sequence of Figure 10.
35	Sequence ID No 27	corresponds to a polypeptide having the amino acid sequence illustrated in Figure 26.
	Sequence ID No 28	corresponds to the sequence from

position 5 to 508 of the nucleotide sequence illustrated in Figure 26.

- | | | |
|----|-------------------|--|
| 5 | Sequence ID No 29 | corresponds to the nucleotide sequence from position 5 to 508 of the nucleotide sequence illustrated in Figure 26. |
| 10 | Sequence ID No 30 | corresponds to the sequence from position 214 to 345 of the nucleotide sequence illustrated in Figure 10. |

[illegible]

CLAIMS

1. A nucleic acid molecule encoding a VEGF-X protein or a functional equivalent, derivative or bioprecursor thereof, said protein comprising any of the sequences from position 23 to 345 of the amino acid sequence illustrated in Figure 10, or the complete sequence as illustrated in Figure 10.
2. A nucleic acid molecule according to claim 1 wherein said nucleic acid is a DNA molecule.
3. A nucleic acid molecule according to claim 1 wherein said nucleic acid is a cDNA molecule.
4. A nucleic acid molecule according to claim 3 comprising the nucleotide sequence from position 257 to 1291 of the nucleotide sequence illustrated in Figure 9, or sequences that hybridise thereto under high stringency conditions or the complement thereto.
5. An antisense molecule capable of hybridising to a molecule according to claim 1 under high stringency conditions.
6. A nucleic acid molecule according to claim 1 which is of mammalian origin.
7. A nucleic acid molecule according to claim 6 which is of human origin.
8. An isolated VEGF-X protein, or a functional equivalent, derivative or bioprecursor thereof, having an amino acid sequence from position 23 to 345 of the amino acid sequence illustrated in Figure 10 or the complete amino acid sequence of Figure 10.

9. A VEGF-X protein, or a functional equivalent, derivative or bioprecursor thereof, encoded by a nucleic acid molecule as defined in claim 1.

5 10. A protein according to claim 9, which comprises the amino acid sequence illustrated in Figure 10.

11. An expression vector comprising a nucleic acid molecule according to claim 1.

10

12. An expression vector according to claim 11 further comprising a nucleotide sequence encoding a reporter molecule.

15 13. An expression vector comprising an antisense molecule according to claim 5.

14. A nucleic acid molecule according to claim 1 for use as a medicament.

20

15. A host cell transformed or transfected with an expression vector according to claim 11 or 12.

25 16. A host cell transformed or transfected with an expression vector according to claim 13.

17. A transgenic cell, tissue or organism comprising a transgene capable of expressing a VEGF-X protein according to claim 8 or 9.

30

18. A transgenic cell, tissue or organism according to claim 17, wherein said transgene is included in an expression vector.

35 19. A VEGF-X protein or a functional equivalent, derivative or bioprecursor thereof, expressed by a cell according to claim 15.

20. A VEGF-X protein, or a functional equivalent, derivative or bioprecursor thereof, expressed by a transgenic cell, tissue or organism according to claim 17.

5

21. A process for producing a VEGF-X protein according to claim 8, said process comprising transforming a host cell or organism with an expression vector according to claim 11, and recovering the expressed protein from said host cell or organism.

10

22. An antibody capable of binding to a protein according to claim 8, or an epitope thereof.

15

23. An antibody according to claim 22 for use as a medicament.

24. A pharmaceutical composition comprising an antibody according to claim 22 together with a pharmaceutically acceptable carrier diluent or excipient thereof.

20

25. A method of identifying VEGF-X protein in a sample which method comprises contacting said sample with an antibody according to claim 22 and monitoring for binding of any protein to said antibody.

25

26. A kit for identifying the presence of VEGF-X protein in a sample which comprises an antibody according to claim 22 and means for contacting said antibody with said sample.

30

27. A method of identifying compounds which modulate angiogenesis which method comprises providing a host cell or organism according to claim 15 or a transgenic cell, tissue or organism according to

35

claim 17, contacting a test compound with said cell,
tissue or organism and monitoring for an effect of
said compound on said VEGF compared to a host cell or
organism according to claim 15 or a transgenic cell
tissue or organism which has not been contacted with
said compound.

28. A compound identifiable according to the method
of claim 27.

29. A compound according to claim 28 for use as a
medicament.

30. A nucleic acid sequence comprising the
nucleotide sequences illustrated in any of Figures 3,
5, 8 or 13.

31. A method for producing a polypeptide, said
method comprising the steps of:

- a) culturing the host cell of claim 15 under
conditions suitable for expression of the
polypeptide; and
- b) recovering the polypeptide from the host
cell culture.

32. A method of inhibiting angiogenic activity and
inappropriate vascularisation including formation and
proliferation of new blood vessels, growth and
development of tissues, tissue regeneration and organ
and tissue repair in a subject said method comprising
administering to said subject an amount of an
antisense molecule according to claim 5 in sufficient
concentration to reduce or prevent said angiogenic
activity.

33. A method of inhibiting angiogenic activity or

inappropriate vascularisation including any of
formation and proliferation of new blood vessels,
growth and development of tissues, tissue
regeneration and organ and tissue repair in a subject
5 said method comprising administering to said subject
an amount of an antibody according to claim 22 in
sufficient concentration to reduce or prevent said
angiogenic activity or inappropriate vascularisation.

10 34. A method of inhibiting angiogenic activity or
inappropriate vascularisation including any of
formation and proliferation of new blood vessels,
growth and development of tissues, tissue
regeneration and organ and tissue repair in a
15 subject, said method comprising implanting in said
subject cells that express an antibody according to
claim 22.

20 35. A method of treating or preventing any of
cancer, rheumatoid arthritis, psoriasis and diabetic
retinopathy, said method comprising administering to
said subject an amount of an antisense molecule
according to claim 5 in sufficient concentration to
treat or prevent said disorders.

25 36. A method of treating or preventing any of
cancer, rheumatoid arthritis, psoriasis and diabetic
retinopathy, said method comprising administering to
said subject an amount of an antibody according to
30 claim 22 in sufficient concentration to reduce or
prevent said disorders.

35 37. A method of promoting angiogenic activity or
vascularisation to promote wound healing, skin graft
growth, tissue repair, proliferation of new blood
vessels, tissue regeneration and organ repair which
method comprises applying or delivering to a site of

interest a therapeutically effective amount of any of
a group selected from a protein according to claim 8
and a nucleic acid molecule encoding a VEGF-X protein
or a functional equivalent, derivative or
5 bioprecursor thereof comprising an amino acid
sequence illustrated in Figure 10, an expression
vector comprising said nucleic acid molecule and a
pharmaceutical composition comprising any of said
nucleic acid molecule and said protein.

10

38. A method of treating wounds selected from the
group consisting of dermal ulcers, pressure sores,
venous sores, diabetic ulcers and burns by applying
to said wound a therapeutically effective amount of
15 any of a VEGF-X protein according to claim 8, a
pharmaceutical composition comprising said protein
and a pharmaceutically acceptable carrier, diluent or
excipient therefor.

20

39. A nucleic acid molecule encoding a polypeptide
having a CUB domain said polypeptide comprising the
amino acid sequence from position 40 to 150 of the
sequence of Figure 10.

25

40. A nucleic acid molecule encoding a polypeptide
having a CUB domain, said polypeptide comprising the
amino acid sequence of Figure 26.

30

41. A nucleic acid molecule according to claim 40,
comprising the nucleotide sequence from position 5 to
508 of the sequence illustrated in Figure 26.

35

42. A nucleic acid molecule according to claim 41
comprising the nucleotide sequence illustrated in
Figure 26.

43. A nucleic acid molecule encoding a VEGF like

domain comprising the sequence from position 214-345 of the sequence of Figure 10 or the sequence from position 15 to 461 illustrated in Figure 24.

- 5 44. An expression vector comprising a nucleic acid molecule according to claim 39 or 40.
45. An expression vector comprising a nucleic acid molecule according to claim 43.
- 10 46. A host cell transformed or transfected with an expression vector according to claim 44.
47. A host cell transformed or transfected with an expression vector according to claim 45.
- 15 48. A protein expressed by the cell according to claim 46.
- 20 49. A protein expressed by the cell according to claim 47.
50. A method of identifying compounds that inhibit or enhance angiogenic activity, said method comprising contacting a cell expressing a VEGF receptor and/or a neuropilin 1 or 2 type receptor with said compound in the presence of a VEGF-X protein according to claim 8 and monitoring for the effect of said compound or said cell when compared to a cell which has not been contacted with said compound.
- 25 51. A compound identifiable according to the method of claim 50 as an inhibitor or enhancer of angiogenic activity.
- 30 52. A method of inhibiting angiogenic activity or
- 35

inappropriate vascularisation, said method comprising contacting a cell expressing a VEGF receptor and a neuropilin type receptor with a protein selected from any of a protein according to claim 8 and a protein according to claim 48 or a protein according to claim 49.

53. Use of a nucleotide sequence illustrated in any of Figures 14 and 15 in identifying a VEGF-X protein according to claim 8.

54. A nucleic acid molecule encoding a polypeptide comprising a CUB domain having the sequence from position 40 to 150 of the sequence of Figure 10 or from position 5 to 508 of the sequence of Figure 26 and a sequence encoding a VEGF domain.

55. A nucleic acid molecule according to claim 54 wherein said sequence encoding said VEGF domain is selected from the sequences encoding any of VEGF A to D or isoforms or variants thereof.

56. A nucleic acid molecule encoding a polypeptide comprising the amino acid sequence from position 40 to 150 of the sequence illustrated in Figure 10 for use as a medicament.

57. Use of a nucleic acid molecule encoding a polypeptide having the amino acid sequence from position 40 to 150 of the sequence illustrated in Figure 10 in the manufacture of a medicament for treatment of disease conditions associated with inappropriate angiogenesis such as tumour or cancer growth, retinopathy, osteoarthritis or psoriasis.

58. A polypeptide comprising the amino acid sequence from position 40 to 150 of the sequence illustrated

in figure 10 for use as a medicament.

59. A polypeptide comprising the amino acid sequence from position 40 to 150 of the sequence illustrated in Figure 10 in the manufacture of a medicament for the treatment of disease conditions associated with inappropriate angiogenesis such as tumour growth, retinopathy, osteoarthritis or psoriasis.

60. Use of a CUB domain comprising the amino acid sequence from position 40 to 150 of the sequence of Figure 10, or the amino acid sequence of Figure 26, to identify compounds which inhibit angiogenic activity in a method according to claim 50.

61. A method of inhibiting angiogenic activity and inappropriate vascularisation including formation and proliferation of new blood vessels, growth and development of tissues, tissue regeneration and organ and tissue repair in a subject said method comprising administering to said subject an amount of a polypeptide having an amino acid sequence from position 40 to 150 of the sequence illustrated in Figure 10 or a nucleic acid molecule according to any of claims 39 to 42 in sufficient concentration to reduce or prevent said angiogenic activity.

62. A method of treating or preventing any of cancer, rheumatoid arthritis, psoriasis and diabetic retinopathy, said method comprising administering to said subject an amount of a polypeptide having an amino acid sequence from position 40 to 150 of the sequence illustrated in Figure 10 or a nucleic acid molecule according to any of claims 39 to 42 in sufficient concentration to treat or prevent said disorders.

63. An antisense molecule capable of hybridising to a molecule according to claim 39 under high stringency conditions.

5 64. An antisense molecule capable of hybridising to a molecule according to claim 43 under high stringency conditions.

10 65. A transgenic cell, tissue or organism comprising a transgene capable of expressing a protein according to claim 48.

15 66. A transgenic cell, tissue or organism comprising a transgene capable of expressing a protein according to claim 49.

20 67. A transgenic, cell tissue or organism according to claim 65 or 66, wherein said transgene is included in an expression vector according to claim 41 or 42.

25 68. An antibody capable of binding to a protein according to claim 48 or an epitope thereof.

30 69. An antibody capable of binding to a protein according to claim 49 or an epitope thereof.

35 70. A pharmaceutical composition comprising an antibody according to claim 68 or 69 together with a pharmaceutically acceptable carrier diluent or excipient therefor.

71. A pharmaceutical composition comprising a compound according to claim 48 together with a pharmaceutically acceptable carrier, diluent or excipient therefor.

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ABSTRACT

VASCULAR ENDOTHELIAL GROWTH FACTOR-X

- 5 There is provided a novel vascular endothelial growth factor, herein designated VEGF-X, in addition to the nucleic acid molecule encoding it, a host cell transformed with said vector and compounds which inhibit or enhance angiogenesis. Also provided is the sequence of a CUB domain present in the sequence of VEGF-X which domain itself prevents angiogenesis and which is used to treat diseases associated with inappropriate vascularisation or angiogenesis.

Separator Sheer



Miscellaneous Material Includes:

- 1. Drawings**
- 2. Oath or Declaration**
- 3. Foreign language specification**
- 4. Sequence listing**
- 5. Computer listing**
- 6. Appendices**

Figure 1.

1 AAAATGTATG GATAACAACCTT ACGTTTGATG AAAGATTGG GCTTGAAGAC CCAGAAGATG
TTTTACATAC CTATGTTGAA TGCAAACCTAC TTTCTAAACC CGAAGCTTCTG GGTCTTCTAC

61 ACATATGCAA GTATGATTTT GTAGAAGTTG AGGAACCCAG TGATGGAAGT ATATTAGGGC
TGTATACGTT CATACTAAAA CATCTTCAAC TCCTTGGGTC ACTACCTTGA TATAATCCCC

121 GCTGGTGTGG TTCTGGTACT GTACCAGGAA AACAGATTTC TAAAGGAAAT CAAATTAGGA
CGACCACACC AAGACCATGA CATGGTCCTT TTGTCTAAAG ATTTCCTTTA GTTTAATCCT

+1 MetAsn IlePheLeu LeuAsnLeuLeu ThrGluGlu ValArgLeu
]-----
181 TAAGATTGTG ATCTGATGAA TATTTTCCTT CTGAACCTTC TAACAGAGGA GGTAAGATTA
ATTCTAAACA TAGACTACTT ATAAAAGGAA GACTTGGAAG ATTGTCTCCT CCATTCTAAT

+1 TyrSerCysThr ProArgAsn PheSerVal SerIleArgGlu GluLeuLys ArgThrAsp

241 TACAGCTGCA CACCTCGTAA CTCTCTCAGTGC TCCATAAGGG AAGAACTAAA GAGAACCGAT
ATGTCGACGT GTGGAGCATT GAAGAGTCAC AGGTATTCCC TTCTTGATTT CTCTTGGCTA

+1 ThrIlePheTrp ProGlyCys LeuLeuVal LysArgCysGly GlyAsnCys AlaCysCys

301 ACCATTTTCT GGCCAGGTTG TCTCCTGGTT AAACGCTGTG GTGGGAACTG TGCCCTGTTGT
TGGTAAAGA CCGGTCCAAC AGAGGACCAA TTTGCGACAC CACCCTTGAC ACGGACAACA

+1 LeuHisAsnCys AsnGluCys GlnCysVal ProSerLysVal ThrLysLys TyrHisGlu

361 CTCCACAATT GCAATGAATG TCAATGTGTC CCAAGCAAAG TTAATAAAAA ATACCACGAG
GAGGTG-TAA CGTTACTTAC AGTTACACAG GGTTCGTTTC AATGATTTTT TATGGTGCTC

+1 ValLeuGlnLeu ArgProLys ThrGlyVal ArgGlyLeuHis LysSerLeu ThrAspVal

421 GTCCTTCACT TGAGACCAAA GACCGGTGTC AGGGGATTGC ACAATCACT CACCGACGTG
CAGGAAGTCA ACTCTGGTTT CTGGCCACAG TCCCCTAACG GTTTTAGTGA GTGGCTGCAC

+1 AlaLeuGluHis HisGluGlu CysAspCys ValCysArgGly SerThrGly Gly
----->
481 GCCCTGGAGC ACCATGAGGA GTGTGACTGT GTGTGCAGAG GGAGCACAGG AGGATAGCCG
CGGGACCTCG TGTTACTCCT CACACTGACA CACACGTCTC CCTCGTGTC TCCTATCGGC

541 CATCACCACC AGCAGCTCTT GCCCAGAGCT GTGCAGTGCA GTGGCTGATT CTATTAGAGA
GTAGTGGTGG TCGTCGAGAA CGGTCTCGA CACGTCACGT CACCGACTAA GATAATCTCT

601 ACGTATGCGT TATCTCCATC CTTAATCTCA GTTGTGTTGCT TCAAGGACCT TTCATCTTCA
TGCATACGCA ATAGAGGTAG GAATTAGAGT CAACAAACGA AGTTCCTGGA AAGTAGAAGT

661 GGATTTACAG TGCATTCTGA AAGAGGAGAC ATCAAACAGA ATTAGGAGTT GTGCAACAGC
CCTAAATGTC ACGTAAGACT TTCTCCTCTG TAGTTTGCT TAATCCTCAA CAGGTTGTGG

721 TCTTTTGAGA GGAGGCCTAA AGGACAGGAG AAAAGGTCTT CAATCGTGGA AAGAAAATTA
AGAAAACCTCT CCTCCGGATT TCCTGTCTC TTTTCCAGAA GTTAGCACCT TTCTTTTAAT

781 AATGTTGTAT TAAATAGATC ACCAGCTAGT TTCAGAGTTA CCATGTACGT ATTCCACTAG
TTACAACATA ATTTATCTAG TGGTCGATCA AAGTCTCAAT GGTACATGCA TAAGGTGATC

841 CTGGGTTCTG TATTTTCAGTT CTTTCGATAC GGCTTAGGGT AATGTCAGTA CAGGAAAAAA
GACCCAAAGAC ATAAAGTCAA GAAAGCTATG CCGAATCCCA TTACAGTCAT GTCCTTTTTT

901 ACTGTGCAAG TGAGCACCTG ATTCCGTTGC CTGCTTAAC TCTAAAGCTC CATGTCTGG
TGACACGTTT ACTCGTGGAC TAAGGCAACG GAACGAATTG AGATTTTCAG GTACAGGACC

961 GCCTAAAAATC GTATAAAATC TGGATTTTTT TTTTTTTTTT TGCTCATATT CACATATGTA
CGGATTTTAG CATATTTTAG ACCTAAAAAA AAAAAAAAAA ACGAGTATAA GTGTATACAT

1021 AACCAGAAAC TTCTATGTAC TACAAACCTG GTTTTTAAAA AGGAACATAG TTGCTATGAA
TTGGTCTTGT AAGATACATG ATGTTTGGAC CAAAAATTTT TCCTTGATAC AACGATACTT

1081 TTAACCTTGT GTCGTGCTGA TAGGACAGAC TGGATTTTTC ATATTCTTTA TTAATAATTC
AATTTGAACA CAGCAGGACT ATCCTGTCTG ACCTAAAAAG TATAAGAAT AATTTTAAAG

1141 TGCCATTTAG AAGAAGAGAA CTACATTCAT GGTTTGGAAG AGATAAACCT GAAAAGAAGA
ACGGTAAATC TTCTTCTCTT GATGTAAGTA CCAAACCTTC TCTATTTGGA CTTTTCTTCT

1201 GTGGCCTTAT CTTCACTTTA TCGATAAGTC AGTTTATTTG TTTCAATTGT TACATTTTAA
CACCAGAAAT GAAGTGAAAT AGCTATTTCAG TCAATAAAC AAAGTAACAC ATGTAAAAAT

1261 TATTCTCCTT TTGACATTAT AACTGTTGGC TTTTCTAATC TTGTTAAATA TATCTATTTT
ATAAGAGGAA AACTGTAATA TTGACAACCG AAAAGATTAG AACAAATTTAT ATAGATAAAA

1321 TACCAAAGGT ATTTAATATT CTTTTTTATG ACAACTTAGA TCAACTATTT TTAGCTTGGT
ATGGTTTCCA TAAATTATAA GAAAAAATAC TGTGAACTCT AGTTGATAAA AATCGAACCA

1381 AAATTTTCTT AAACACAATT GTTATAGCCA GAGGAACAAA GATGATATAA AATATTGTTG
TTTAAAAAGA TTTGTGTTAA CAATATCGGT CTCCTTGTTT CTACTATATT TTATAACAAC

1441 CTCTGACAAA AATACATGTA TTTCATTCTC GTATGGTGCT AGAGTTAGAT TAATCTGCAT
GAGACTGTTT TTATGTACAT AAAGTAAGAG CATACCACGA TCTCAATCTA ATTAGACGTA

1501 TTTAAAAAAC TGAATTGGAA TAGAATTGGT AAGTTGCAAA GACTTTTIGA AAATAATTAA
AAATTTTTTG ACTTAACCTT ATCTTAACCA TTCAACGTTT CTGAAAAACT TTTATTAATT

1561 ATTATCATAT CTTCCATTCC TGTATTGGA GATGAAAATA AAAAGCAACT TATGAAAGTA
TAATAGTATA GAAGTAAGG ACAATAACCT CTACTTTTAT TTTTCGTTGA ATACTTTTAT

1621 GACATTCAGA TCCAGCCATT ACTAACCTAT TCCTTTTTTG GGGAAATCTG AGCCTAGCTC
CTGTAACTCT AGGTCCGTAA TGATTGGATA AGGAAAAAAC CCCTTTAGAC TCGGATCGAG

1681 AGAAAAACAT AAGCACCTT GAAAAAGACT TGGCAGCTTC CTGATAAAGC GTGCTGTGCT
TCTTTTGTG TTTCTGTGAA CTTTTTCTGA ACCGTGGAAG GACTATTTCT CACGACACGA

1741 GTGCAGTAGG AACACATCCT ATTTATTGTG ATGTTGTGGT TTTATTATCT TAACTCTGT
CACGTCATCC TTGTGTAGGA TAAATAACAC TACAACACCA AAATAATAGA ATTTGAGACA

1801 TCCATACACT TGTATAATA CATGGATATT TTTATGTACA GAAGTATGTC TCTTAACCA
AGGTATGGA ACATATTTAT GTACCTATAA AAATACATGT CTTCATACAG AGAATTGGTC

Fig. 1 (cont.)

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AAGTGAATAA CATGGACC

Fig. 1 (cont.)

Figure 2. Predicted VEGF-like protein encoded by Incyte contig of 8/12/98

1 MNIFLLNLLT EEVRLYSCTP RNFSVSIREE LKRTDTIFWP GCLLVKRCGG
51 NCACCLHNCN ECQCVPSKVT KKYHEVLQLR PKTGVRGLHK SLTDVALEHH
101 EECDCVCRGS TGG

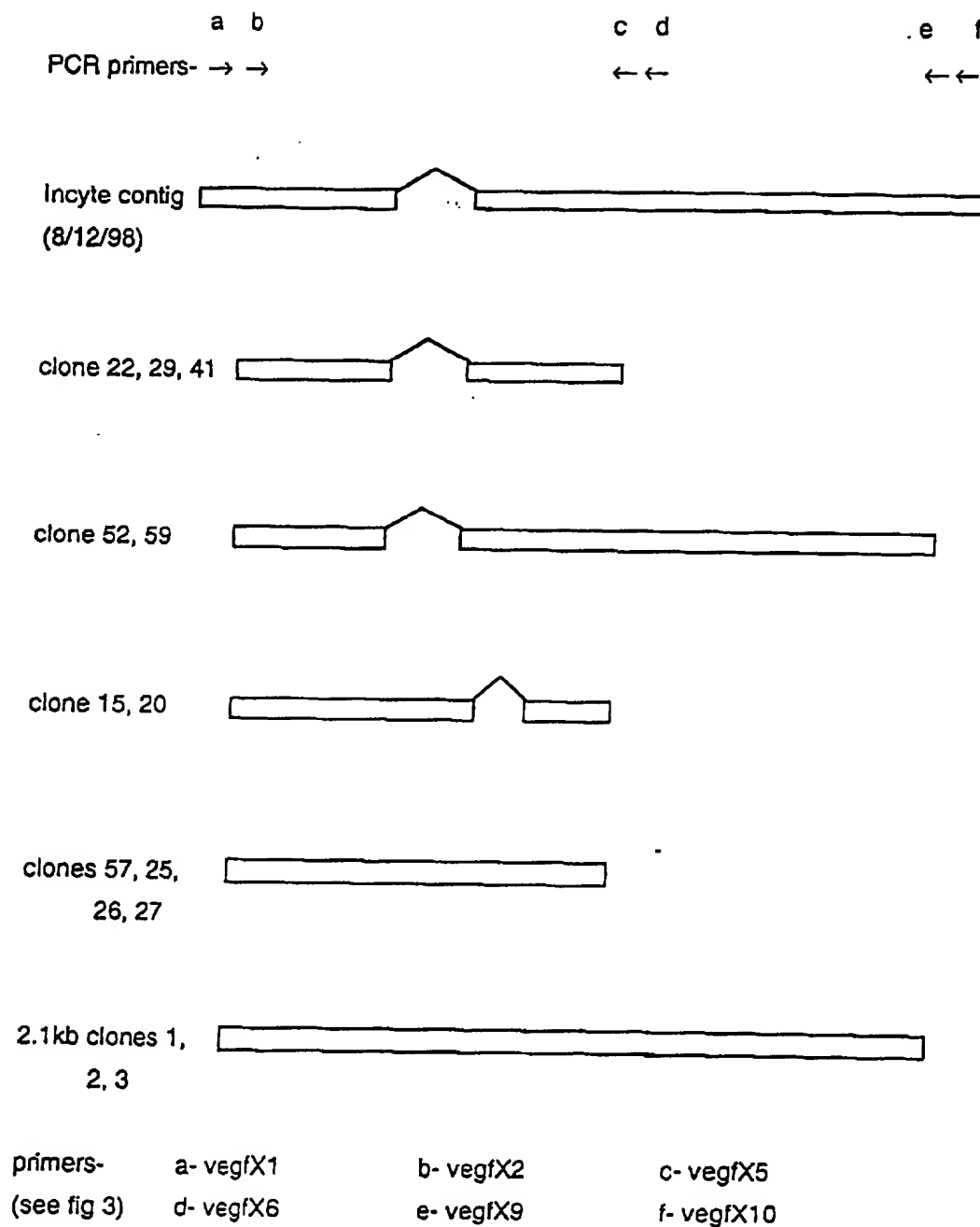
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Figure 3. PCR primers for cloning VEGF-X

vegfx1	AAAATGTATGGATACAACTTAC
vegfx2	GTTTGATGAAAGATTGGGCTTG
vegfx3	TTTCTAAAGGAAATCAAATTAG
vegfx4	GATAAGATTGTATCTGATG
vegfx5	GATGTCTCCTCTTTCAG
vegfx6	GCACAACTCCTAATTCTG
vegfx7	AGCACCTGATTCCGTTGC
vegfx8	TAGTACATAGAATGTTCTGG
vegfx9	AAGAGACATACTTCTGTAC
vegfx10	CCAGGTACAATAAGTGAAGTCTG

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Figure 4. Variants Isolated by PCR



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Figure 5. VEGF-X 5' RACE primers

vegX11	CCTTTAGAAATCTGTTTTCTGGTACAG
vegX12	GGAAAATATTCATCAGATACAAATCTTATCC
vegX13	GGTCCAGTGGCAAAGCTGAAGG
vegX14	CTGGTTCAAGATATCGAATAAGGTCTTCC

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Figure 6. DNA sequence assembled from in-house clones and 5'RACE

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1  TGCCAGAGCA GGTGGGCGCT TCCACCCAG TGCAGCCTTC CCCTGGCGGT GGTGAAAGAG
   ACGGTCTCGT CCACCCGCGA AGGTGGGGTC ACGTCGGAAG GGGACCGCCA CCACTTTCTC

61  ACTCGGGAGT CGCTGCTTCC AAAGTGCCCG CCGTGAGTGA GCTCTCACCC CAGTCAGCCA
   TGAGCCCTCA GCGACGAAGG TTTCACGGGC GGCACCTCACT CGAGAGTGGG GTCAGTCGGT

+2  MetSerLeu PheGlyLeuLeu LeuLeuThr SerAlaLeu AlaGlyGlnArg GlnGlyTh
   ]-----
121  AATGAGCCTC TTCGGGCTTC TCCTGCTGAC ATCTGCCCTG GCCGGCCAGA GACAGGGGAC
   TTACTCGGAG AAGCCCGAAG AGGACGACTG TAGACGGGAC CGGCCGGTCT CTGTCCCTG

+2  rGlnAlaGlu SerAsnLeuSer SerLysPhe GlnPheSer SerAsnLysGlu GlnAsnGl
   -----
181  TCAGGCGGAA TCCAACCTGA GTAGTAAATT CCAGTTTTC AGCAACAAGG AACAGAACGG
   AGTCCGCCTT AGGTTGGACT CATCATTTAA GGTCAAAAGG TCGTTGTTC TTGTCTGCC

+2  yValGlnAsp ProGlnHisGlu ArgIleIle ThrValSer ThrAsnGlySer IleHisSe
   -----
241  AGTACAAGAT CCTCAGCATG AGAGAATTAT TACTGTGTCT ACTAATGGAA GTATTCACAG
   TCATGTCTTA GGAGTCGTAC TCTCTTAATA ATGACACAGA TGATTACCTT CATAAGTGTC

+2  rProArgPhe ProHisThrTyr ProArgAsn ThrValLeu ValTrpArgLeu ValAlaVa
   -----
301  CCCAAGGTTT CCTCATACTT ATCCAAGAAA TACGGTCTTG GTATGGAGAT TAGTAGCAGT
   GGGTTCCAA GGAGTATGAA TAGGTTCTTT ATGCCAGAAC CATACCTCTA ATCATCGTCA

+2  lGluGluAsn ValTrpIleGln LeuThrPhe AspGluArg PheGlyLeuGlu AspProGl
   -----
361  AGAGGAAAAT GTATGGATAC AACTTACGTT TGATGAAAGA TTTGGGCTTG AAGACCCAGA
   TCTCCTTTTA CATACCTATG TTGAATGCAA ACTACTTTCT AAACCCGAAC TTCTGGGTCT

+2  uAspAspIle CysLysTyrAsp PheValGlu ValGluGlu ProSerAspGly ThrIleLe
   -----
421  AGATGACATA TGCAAGTATG ATTTTGTAGA AGTTGAGGAA CCCAGTGATG GAACTATATT
   TCTACTGTAT ACGTTCATAC TAAACATCT TCAACTCCTT GGGTCACTAC CTTGATATAA

+2  uGlyArgTrp CysGlySerGly ThrValPro GlyLysGln IleSerLysGly AsnGlnIl
   -----
481  AGGGCGCTGG TGTGTTCTG GTACTGTACC AGGAAAACAG ATTTCTAAAG GAAATCAAAT
   TCCCGCGACC ACACCAAGAC CATGACATGG TCCTTTTGTG TAAAGATTTT CTTTAGTTTA

+2  eArgIleArg PheValSerAsp GluTyrPhe ProSerGlu ProGlyPheCys IleHisTy
   -----
541  TAGGATAAGA TTTGTATCTG ATGAATATT TCCTTCTGAA CCAGGGTTCT GCATCCACTA
   ATCTATTCT AACATAGAC TACTTATAAA AGGAAGACTT GGTCCCAAGA CGTAGGTGAT

+2  rAsnIleVal MetProGlnPhe ThrGluAla ValSerPro SerValLeuPro ProSerAl
   -----
601  CAACATTSTC ATGCCACAAT TCACAGAAGC TGTGAGTCCT TCAGTGCTAC CCCCTTCAGC
   GTGTAAACAG TACGGGTGTA AGTGCTCTCG ACATCAGGA AGTCACGATG GGGGAAGTCG

+2  aLeuProLeu AspLeuLeuAsn AsnAlaIle ThrAlaPhe SerThrLeuGlu AspLeuIl
   -----

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Fig. 6 (cont.)

661 TTGCCACTG GACCTGCTTA ATAATGCTAT AACTGCCTTT AGTACCTTGG AAGACCTTAT
AAACGOTGAC CTGGACGAAT TATTACGATA TTGACGGAAA TCATGGAAAC TTCTGGAATA

+2 eArgTyrLeu GluProGluArg TrpGlnLeu AspLeuGlu AspLeuTyrArg ProThrTr

721 TCGATATCTT GAACCAGAGA GATGGCAGTT GGACTTAGAA GATCTATATA GGCCAACTTG
AGCTATAGAA CTGGTCTCT CTACCGTCAA CCTGAATCTT CTAGATATAT CCGGTGAAC

+2 pGlnLeuLeu GlyLysAlaPhe ValPheGly ArgLysSer ArgValValAsp LeuAsnLe

781 GCAACTTCTT GGCAAGGCTT TTGTTTTTGG AAGAAAATCC AGAGTGGTGG ATCTGAACCT
CGTTGAAGAA CCGTTCCGAA AACAAAAACC TTCTTTTAGG TCTCACCACC TAGACTTGA

+2 uLeuThrGlu GluValArgLeu TyrSerCys ThrProArg AsnPheSerVal SerIleAr

841 TCTAACAGAG GAGGTAAAGAT TATACAGCTG CACACCTCGT AACTTCTCAG TGTCCATAAG
AGATTGTCTC CTCCATTCTA ATATGTCGAC GTGTGGAGCA TTGAAGAGTC ACAGGTATTC

+2 gGluGluLeu LysArgThrAsp ThrIlePhe TrpProGly CysLeuLeuVal LysArgCy

901 GGAAGAACTA AAGAGAACCG ATACCATTTT CTGGCCAGGT TGTCTCCTGG TTAAACGCTG
CCTTCTTGAT TTCTCTTGGC TATGTTAAAA GACCGGTCCA ACAGAGGACC AATTTGCGAC

+2 sGlyGlyAsn CysAlaCysCys LeuHisAsn CysAsnGlu CysGlnCysVal ProSerLy

961 TGGTGGGAAC TGTGCCTGTT GTCTCCACAA TTGCAATGAA TGTCAATGTG TCCCAAGCAA
ACCACCCTTG ACACCGACAA CAGAGGTGTT AACGTACTT ACAGTTACAC AGGGTTCGTT

+2 sValThrLys LysTyrHisGlu ValLeuGln LeuArgPro LysThrGlyVal ArgGlyLe

1021 AGTTACTAAA AAATACCACG AGGTCCTTCA GTTGAGACCA AAGACCGGTG TCAGGGGATT
TCAATGATTT TTTATGGTGC TCCAGGAAGT CAACTCTGST TTCTGGCCAC AGTCCCCTAA

+2 uHisLysSer LeuThrAspVal AlaLeuGlu HisHisGlu GluCysAspCys ValCysAr

1081 GCACAAATCA CTCACCGACG TGGCCCTGGA GCACCATGAG GAGTGTGACT GTGTGTGCAG
CGTGTTTAGT GAGTGGCTGC ACCGGGACCT CGTGGTACTC CTCACACTGA CACACACGTC

+2 gGlySerThr GlyGly
----->

1141 AGGGAGCACA GGAGGATAGC CGCATCACCA CCAGCAGCTC TTGCCCAGAG CTGTGCAGTG
TCCCTCGTGT CCTCCTATCG GCGTAGTGGT GGTCTGTCGAG AACGGGTCTC GACACGTCAC

1201 CAGTGGCTGA TTCTATTAGA GAACGTATGC GTTATCTCCA TCCTTAATCT CAGTTGTTTG
GTCACCGACT AAGATAATCT CTGTCATACG CAATAGAGGT AGGAATTAGA GTCAACAAAC

1261 CTTCAAGGAC CTTTCATCTT CAGGATTTAC AGTGCATTCT GAAAGAGGAG ACATCAAACA
GAAGTTCCTG GAAAGTAGAA GTCCTAAATG TCACGTAAGA CTTTCTCCTC TGTAGTTTGT

1321 GAATTAGGAG TTGTGCAACA GCTCTTTTGA GAGGAGGCCT AAAGGACAGG AGAAAAGGTC
CTTAATCCTC AACACGTTGT CGAGAAAACT CTCCTCCGGA TTCTCTGTCC TCTTTTCCAG

1381 TTCAATCTTG GAAAGAAAAT TAAATGTTGT ATTAAATAGA TCACCAGCTA GTTTCAGAGT
AAGTTATCAC CTTTCTTTTA ATTTACAACA TAATTATCT AGTGGTCGAT CAAAGTCTCA

1441 TACCATGTAC GTATTCCACT AGCTGGGTTT TGTATTTTCTG TTCTTTCTGAT ACGGCTTAGG

661 721 781 841 901 961 1021 1081 1141 1201 1261 1321 1381 1441

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Fig 6 (cont.)

ATGGTACATG CATAAGGTGA TCGACCCAAG ACATAAAGTC AAGAAAGCTA TGCCGAATCC

1501 GTAATGTCAG TACAGGAAAA AAACGTGTGCA AGTGAGCACC TGATTCCGTT GCCTTGCTTA
CATTACAGTC ATGTCCTTTT TTTGACACGT TCACTCGTGG ACTAAGGCAA CGGAACGAAT

1561 ACTCTAAAGC TCCATGTCCT GGGCCTAAAA TCGTATAAAA TCTGGATTTT TTTTTTTTTT
TGAGATTTG AGGTACAGGA CCCGATTTT AGCATATTTT AGACCTAAAA AAAAAAAAAA

1621 TTTGCTCATA TTCACATATG TAAACCAGAA CATTCTATGT ACTACAAACC TGGTTTTTAA
AAACGAGTAT AAGTGATATC ATTTGGTCTT GTAAGATACA TGATGTTTGG ACCAAAAAT

1681 AAAGGAACCTA TGTGCTATG AATTAACTT GTGTCGTGCT GATAGGACAG ACTGGATTTT
TTCCCTTGAT ACAACGATAC TTAATTTGAA CACAGCACGA CTATCCTGTC TGACCTAAAA

1741 TCATATTTCT TATTAAAAAT TCTGCCATTT AGAAGAAGAG AACTACATTC ATGGTTTGGA
AGTATAAGA ATAATTTTAA AGACGGTAAA TCTTCTTCTC TTGATGTAAG TACCAAACTT

1801 AGAGATAAAC CTGAAAAGAA GAGTGGCCTT ATCTTCACTT TATCGATAAG CCAGTTTATT
TCTCTATTTG GACTTTTCTT CTCACCGGAA TAGAAGTGAA ATAGCTATTC GGTCAAATAA

1861 TGTTTCATTTG TGTACATTTT TATATTCTCC TTTTGACATT ATAAGTGTG GCTTTTCTAA
ACAAAGTAAC ACATGTAATA ATATAAGAGG AAAACTGTAA TATTGACAAC CGAAAAGATT

1921 TCTTGTTAAA TATATCTATT TTTACCAAGG GTATTTAATA TTCTTTTTTA TGACAACTTA
AGAACAATTT ATATAGATAA AAATGGTTTC CATAATTAT AAGAAAAAT ACTGTTGAAT

1981 GATCAACTAT TTTTAGCTTG GTAAATTTTT CTAACACAA TTGTTATAGC CAGAGGAACA
CTAGTTGATA AAAATCGAAC CATTAAAAA GATTTGTGTT AACAAATATCG GTCTCCTTGT

2041 AAGATGATAT AAAATATTGT TGCTCTGACA AAAATACATG TATTTTATTC TCGTATGGTG
TTCTACTATA TTTTATAACA ACGAGACTGT TTTTATGTAC ATAAAGTAAG AGCATACCAC

2101 CTAGAGTAG ATTAATCTGC ATTTTAAAAA ACTGAATTGG AATAGAATTG GTAAGTTGCA
GATCTCAATC TAATTAGACG TAAAATTTT TGAATTAACC TTATCTTAAC CATTCAACGT

2161 AAGACTTTTT GAAAAATAAT AAATTATCAT ATCTTCCATT CCTGTTATTG GAGATGAAAA
TTCTGAAAAA CTTTTATTAA TTTAATAGTA TAGAAGGTAA GGACAATAAC CTCTACTTTT

2221 TAAAAAGCAA CTTATGAAAG TAGACATTCA GATCCAGCCA TTACTAACCT ATTCCTTTTT
ATTTTTCGTT GAATACTTTC ATCTGTAAGT CTAGGTCGGT AATGATTGGA TAAGGAAAAA

2281 TGGGGAAATC TGAGCCTAGC TCAGAAAAAC ATAAAGCACC TTGAAAAAGA CTTGGCAGCT
ACCCCTTTAG ACTCGGATCG AGTCTTTTTG TATTTCTGTT AACTTTTTCT GAACCGTCGA

2341 TCCTGATATA GCGTGCTGTG CTGTGCAGTA GGAACACATC CTATTTATTG TGATGTTGTG
AGGACTATTT CGCAGCACAC GACACGTCAT CCTGTGTAG GATAAATAAC ACTACAACAC

2401 GTTTTATAT CTTAACTCT GTTCCATACA CTTGTATAAA TACATGGATA TTTTATGTA
CAAAATAATA GAATTTGAGA CAAGGTATGT GAACATATTT ATGTACCTAT AAAAAATACAT

2461 CAGAAGTATG TCTCT
GTCTTCAATC AGAGA

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Figure 7. New Sequence + Incyte ESTs

1 ATTTGTTTAA ACCTTGGGAA ACTGGTTCAG GTCCAGGTTT TGCTTTGATC CTTTTCAAAA
TAAACAAATT TGGAAACCTT TGACCAAGTC CAGGTCCAAA ACGAAACTAG GAAAAAGTTT

61 ACTGGAGACA CAGAAGAGGG CTTCTAGGAA AAAGTTTGG GATGGGATTA TGTGGAAACT
TGACCTCTGT GTCTTCTCCC GAAGATCCTT TTTCAAAACC CTACCCTAAT ACACCTTTGA

121 ACCCTGCGAT TCTCTGCTGC CAGAGCAGGC TCGGCGCTTC CACCCCAAGT CAGCCTTCCC
TGGGACGCTA AGAGACGACG GTCTCGTCCG AGCCGCGAAG GTGGGGTCAC GTCGGAAGGG

181 CTGGCGGTGG TGAAAGAGAC TCGGGAGTCG CTGCTTCCAA AGTGCCCGCC GTGAGTGAGC
GACCGCCACC ACTTCTCTG AGCCCTCAGC GACGAAGGT TCACGGGCGG CACTCACTCG

+2 Met SerLeuPhe GlyLeuLeu LeuLeuThrSer AlaLeuAl
]-----

241 TCTCACCCCA GTCAGCCAAA TGAGCCTCTT CGGGCTTCTC CTGCTGACAT CTGCCCTGGC
AGAGTGGGGT CAGTCGGTTT ACTCGGAGAA GCCCAAGAG GACGACTGTA GACGGGACCG

+2 aGlyGlnArg GlnGlyThrGln AlaGluSer AsnLeuSer SerLysPheGln PheSerSe

301 CGGCCAGAGA CAGGGGACTC AGGCGGAATC CAACCTGAGT AGTAAATTCC AGTTTTCAG
GCCGGTCTCT GTCCCTGAG TCCGCCTTAG GTTGGACTCA TCATTAAAGG TCAAAAGGTC

+2 rAsnLysGlu GlnTyrGlyVal GlnAspPro GlnHisGlu ArgIleIleThr ValSerTh

361 CAACAAGGAA CAGTACGGAG TACAAGATCC TCAGCATGAG AGAATTATTA CTGTGTCTAC
GTTGTTCCTT GTCATGCCTC ATGTTCTAGG AGTCGTACTC TCTTAATAAT GACACAGATG

+2 rAsnGlySer IleHisSerPro ArgPhePro HisThrTyr ProArgAsnThr ValLeuVa

421 TAATGGAGT ATTACAGCC CAAGGTTTCC TCATACTTAT CCAAGAAATA CGGTCTTGGT
ATTACCTTCA TAAGTGTGG GTTCCAAAGG AGTATGAATA GGTCTTTAT GCCAGAACCA

+2 lTrpArgLeu ValAlaValGlu GluAsnVal TrpIleGln LeuThrPheAsp GluArgPh

481 ATGGAGATTA GTAGCAGTAG AGGAAATGT ATGGATACAA CTTACGTTTG ATGAAAGATT
TACCTCTAAT CATCGTCATC TCCTTTTACA TACCTATGTT GAATGCAAAC TACTTTCTAA

+2 eGlyLeuGlu AspProGluAsp AspIleCys LysTyrAsp PheValGluVal GluGluPr

541 TGGGCTTGAA GACCCAGAAG ATGACATATG CAAGTATGAT TTTGTAGAAG TTGAGGAACC
ACCCGAACCT CTGGGTCTTC TACTGTATAC GTTCATACTA AAACATCTTC AACTCCTTGG

+2 oSerAspGly ThrIleLeuGly ArgTrpCys GlySerGly ThrValProGly LysGlnIl

601 CAGTGATGGA ACTATATTAG GGCGCTGGTG TGTTCTGGT ACTGTACCAG GAAAACAGAT
GTCACTACCT TGATATAATC CCGCGACCAC ACCAAGACCA TGACATGGTC CTTTGTCTA

+2 eSerLysGly AsnGlnIleArg IleArgPhe ValSerAsp GluTyrPhePro SerGluPr

661 TTCTAAAGGA AATCAAATTA GGATAAGATT TGTATCTGAT GAATATTTTC CTTCTGAACC
AAGATTTCCT TTAGTTTAAT CCTATTCTAA ACATAGACTA CTTATAAAAG GAAGACTTGG

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Fig. 7 (cont.)

+2 oGlyPheCys IleHisTyrAsn IleValMet ProGlnPhe ThrGluAlaVal SerProSe

721 AGGGTTCCTGC ATCCACTACA ACATTGTCAT GCCACAATTC ACAGAAGCTG TGAGTCCTTC
TCCCAAGACG TAGGTGATGT TGTAAACAGTA CGGTGTTAAG TGTCTTCGAC ACTCAGGAAG

+2 rValLeuPro ProSerAlaLeu ProLeuAsp LeuLeuAsn AsnAlaIleThr AlaPheSe

781 AGTGCTACCC CCTTCAGCTT TGCCACTGGA CCTGCTTAAT AATGCTATAA CTGCCTTTAG
TCACGATGGG GGAAGTCGAA ACGGTGACCT GGACGAATTA TTACGATATT GACGGAAATC

+2 rThrLeuGlu AspLeuIleArg TyrLeuGlu ProGluArg TrpGlnLeuAsp LeuGluAs

841 TACCTTGGAA GACCTTATTC GATATCTTGA ACCAGAGAGA TGGCAGTTGG ACTTAGAAGA
ATGGAACTTT CTGGAATAAG CTATAGAACT TGGTCTCTCT ACCGTCACCC TGAATCTTCT

+2 pLeuTyrArg ProThrTrpGln LeuLeuGly LysAlaPhe ValPheGlyArg LysSerAr

901 TCTATATAGG CCAACTTGGC AACTTCTTGG CAAGGCTTTT GTTTTGGAA GAAATCCAG
AGATATATCC GGTGAACCG TTGAAGAACC GTCCGAAAA CAAAAACCTT CTTTATAGTC

+2 gValValAsp LeuAsnLeuLeu ThrGluGlu ValArgLeu TyrSerCysThr ProArgAs

961 AGTGGTGSAT CTGAACCTTC TAACAGAGGA GGTAAGATTA TACAGCTGCA CACCTCGTAA
TCACCACCTA GACTTGAAG ATTGTCTCCT CCATTCTAAT ATGTGCGCGT GTGGAGCATT

+2 nPheSerVal SerIleArgGlu GluLeuLys ArgThrAsp ThrIlePheTrp ProGlyCy

1021 CTCTCTAGTG TCCATAAGGG AAGAACTAAA GAGAACCGAT ACCATTTTCT GGCCAGGTTG
GAAGAGTCAC AGGTATTCCC TTCTTGATTT CTCTGGCTA TGGTAAAGA CCGGTCCAAC

+2 sLeuLeuVal LysArgCysGly GlyAsnCys AlaCysCys LeuHisAsnCys AsnGluCy

1081 TCTCCTGGTT AAACGCTGTG GTGGGAACGT TGCCTGTTGT CTCCACAATT GCAATGAATG
AGAGGACCAA TTTGCGACAC CACCCTTGAC ACGGACAACA GAGGTGTAA CGTTACTTAC

+2 sGlnCysVal ProSerLysVal ThrLysLys TyrHisGlu ValLeuGlnLeu ArgProLy

1141 TCAATGTCTC CCAAGCAAAG TTAATAAAAA ATACCACGAG GTCCTTCAGT TGAGACCAAA
AGTTACACAG GGTTCGTTTC AATGATTTTT TATGGTGCTC CAGGAAGTCA ACTCTGGTTT

+2 sThrGlyVal ArgGlyLeuHis LysSerLeu ThrAspVal AlaLeuGluHis HisGluGl

1201 GACCGGTGTC AGGGGATTGC ACAAATCACT CACCGACGTG GCCCTGGAGC ACCATGAGGA
CTGGCCACAG TCCCCTAACG TGTTTAGTGA GTGGCTGCAC CGGGACCTCG TGGTACTCCT

+2 uCysAspCys ValCysArgGly SerThrGly Gly
----->
1261 GTGTGACTGT GTGTGCAGAG GGAGCACAGG AGGATAGCCG CATCACCACC AGCAGCTCTT
CACACTGACA CACACGTCTC CTCGTGTCC TCCTATCGGC GTAGTGGTGG TCGTCGAGAA

1321 GCCCAGACCT GTGCAGTGCA GTGGCTGATT CTATTAGAGA ACGTATGCGT TATCTCCATC
CGGGTCTGCA CACGTACAGT CACCGACTAA GATAATCTCT TGCATACGCA ATAGAGGTAG

1391 CTTAATCTCA GTTGTGCT TCAAGGACCT TTCATCTTCA GGATTACAG TGCATTCTGA
GAATTAGAGT CAACAAACGA AGTTCCTGGA AATAGAAAGT CCTAAATGTC ACCTAAGACT

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Fig. 7 (cont.)

1441 AAGAGGAGAC ATCAAACAGA ATTAGGAGTT GTGCAACAGC TCTTTTGAGA GGAGGCGZAA
 TTCTCCTCTG TAGTTTGTCT TAATCCTCAA CACGTTGTCTG AGAAAACCTCT CCTCCGGATT
 1501 AGGACAGGAG AAAAGGTCTT CAATCGTGGG AAGAAAATTA AATGTTGTAT TAAATAGATC
 TCCTGICCTC TTTTCCAGAA GTTAGCACCT TTCTTTTAAT TTACAACATA ATTTATCTAG
 1561 ACCAGCTAGT TTCAGAGTTA CCATGTACGT ATTCCACTAG CTGGGTTCTG TATTTCASTT
 TGGTCTGATCA AAGTCTCAAT GGTACATGCA TAAGGTGATC GACCCAAGAC ATAAAGTCAA
 1621 CTTTCGATAC GGCTTAGGGT AATGTCAGTA CAGGAAAAAA ACTGTGCAAG TGAGCACCTG
 GAAAGCTATG CCGAATCCCA TTACAGTCAT GTCCTTTTTT TGACACGTTT ACTCGTGGAC
 1691 ATTCCGTTGC CTTGGCTTAA CTCTAAAGCT CCATGTCCTG GGCCTAAAAT CGTATAAAAT
 TAAGGCAACG GAACCGAATT GAGATTTTGA GGTACAGGAC CCGGATTTTA GCATATTTTA
 1741 CTGGATTTTT TTTTTTTTTT TTGCGCATAT TCACATATGT AAACCAGAAC ATCTATGTA
 GACCTAAAAA AAAAAAAAAA AACGCGTATA AGTGTATACA TTTGGTCTTG TAAGATACAT
 1801 CTACAAACCT GGTTTTTTAA AAGGAACTAT GTTGCTATGA ATTAAACTTG TGTCATGCTG
 GATGTTTGA CCAAAATTT TTCCTTGATA CAACGATACT TAATTTGAAC ACAGTACGAC
 1861 ATAGGACAGA CTGGATTTTT CATATTTCTT ATTAAATTT CTGCCATTTA GAAGAAGAGA
 TATCCTGTCT GACCTAAAAA GTATAAGAA TAATTTTAAA GACGGTAAAT CTCTTCTCT
 1921 ACTACATTCA TGGTTTGGAA GAGATAAACC TGAAGAAGAG AGTGGCCTTA TCTTCACTTT
 TGATGTAAAT ACCAAACCTT CTCTATTTGG ACTTTTCTTC TCACCGGAAT AGAAGTGAAA
 1981 ATCGATAAGT CAGTTTATTT GTTTCATTGT GTACATTTTT ATATTCTCCT TTTGACATTA
 TAGCTATTCA GTCAAATAAA CAAAGTAACA CATGTAAAAA TATAAGAGGA AAAGTGAAT
 2041 TAACTGTTGG CTTTCTAAT CTGTATAAT ATATCTATTT TTACCAAAGG TATTTAATAT
 ATTGACACC GAAAAGATTA GAACAATTTA TATAGATAAA AATGGTTTCC ATAAATTATA
 2101 TCTTTTTTAT GACAACTTAG ATCAACTATT TTTAGCTTGG TAAATTTTTT TAAACACAAT
 AGAAAAATA CTGTTGAATC TAGTTGATAA AAATCGAACC ATTTAAAAAG ATTTGTGTTA
 2161 TGTATAGCC AGAGGAACAA AGATGATATA AAATATTGTT GCTCTGACAA AAATACATGT
 ACAATAACCG TCTCCTGTT TCTACTATAT TTTATAACAA CGAGACTGTT TTTATGTACA
 2221 ATTTCAATCT CGTATGGTGC TAGAGTTAGA TTAATCTGCA TTTTAAAAAA CTGAATTGGA
 TAAAGTAAGA GCATACCACG ATCTCAATCT AATTAGACGT AAAATTTTTT GACTTAACCT
 2281 ATAGAATTGG TAAGTTGCAA AGACTTTTTG AAAATAATTA AATTATCTA TCTTCCATTC
 TATCTTAACC ATTCAACGTT TCTGAAAAAC TTTTATTAAT TTAATAGTAT AGAAGGTAAG
 2341 CTGTTATTGG AGATGAAAAT AAAAGCAAC TTATGAAAGT AGACATTGAG ATCCAGCCAT
 GACAATAACC TCTACTTTTA TTTTTCGTTG AATACTTTCA TCTGTAAGTC TAGGTGCGTA
 2401 TACTAACCTA TTCCTTTTTT GGGGAAATCT GAGCCTAGCT CAGAAAAACA TAAAGCACCT
 ATGATTGGAT AAGGAAAAAA CCCCTTTAGA CTCGGATCGA GTCTTTTTGT ATTTGCTGGA
 2461 TGAAGGAGAC TTGGCAGCTT CCGATAAAG CGTGCTGTGC TGTGCACTAG GAACACATCC
 ACTTTTTCTG AACCGTCGAA GGACTATTTT GCACGACACG ACACGTCATC CTTGTGTAGG
 2521 TATTTATTGT GATGTTGTGG TTTTATTATC TTAAGCTCTG TTCCATACAC TTGTATAAAT
 ATAAATAACA CTACACACC AAAATAATAG AATTGAGAC AAGGTATGTG AACATATTTA

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Fig. 7. (cont.)

2581 ACATGGATAT TTTTATGTAC AGAAGTATGT CTCTTAACCA GTTCACTTAT TGTACTCTGG
TGTACCTATA AAAATACATG TCTTCATACA GAGAATTGGT CAAAGTGAATA ACATGAGACC

2641 CAATTTAAAA GAAAATCAGT AAAATATTTT GCTTGTAATA TGCTTAATAT CGTGCCTAGG
GTTAAATTTT CTTTTAGTCA TTTTATAAAA CGAACATTTT ACGAATTATA GCACGGATCC

2701 TTATGTGGTG ACTATTTGAA TCAAAAATGT ATTGAATCAT CAAATAAAAG AATGTGGCTA
AATACACCAC TGATAAACTT AGTTTTTACA TAACTTAGTA GTTTATTTTC TTACACCGAT

2761 TTTTGGGGAG AAAATT
AAAACCCCTC TTTTAA

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5'-2 GTCCAGGTTTTGCTTTGATCC

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Figure 9. DNA Sequence Of Clones 4 & 7, Identical Clones Containing The Entire Open Reading Frame

```

1  TTTGTTTAAA CCTTGGGAAA CTGGTTCAGG TCCAGGTTTT GCTTTGATCC TTTTCAAAA
   AAACAAATTT GGAACCCCTT GACCAAGTCC AGGTCCAAAA CGAAACTAGG AAAAGTTTTT

61  CTGGAGACAC AGAAGAGGGC TCTAGGAAAA AGTTTTGGAT GGGATTATGT GGAAACTACC
   GACCTCTGTG TCTTCTCCCG AGATCCTTTT TCAAAACCTA CCCTAATACA CCTTTGATGG

121 CTGCGATTCT CTGCTGCCAG AGCAGGCTCG GCGCTTCCAC CCCAGTGCAG CCTTCCCCTG
   GACGCTAAGA GACGACGGTC TCCTCCGAGC CGCGAAGGTG GGGTCACGTC GGAAGGGGAC

181 GCGGTGCTGA AAGAGACTCG GGAGTCGCTG CTTCCAAAGT GCCCGCCGTG AGTGAGCTCT
   CGCCACCACT TTCTCTGAGC CCTCAGCGAC GAAGGTTTCA CGGGCCGCAC TCACTCGAGA

+2                               MetSer LeuPheGly LeuLeuLeu LeuThrSerAla LeuAlaGl
   ]-----

241 CACCCAGTC AGCCAAATGA GCCTCTTCGG GCTTCTCCTG CTGACATCTG CCCTGGCCGG
   GTGGGGTCAG TCGGTTTACT CGGAGAAGCC CGAAGAGGAC GACTGTAGAC GGGACCGGGC

+2 yGlnArgGln GlyThrGlnAla GluSerAsn LeuSerSer LysPheGlnPhe SerSerAs
   -----

301 CCAGAGACAG GGGACTCAGG CGGAATCCAA CCTGAGTAGT AAATTCCAGT TTTCCAGCAA
   GGTCTCTGTC CCCTGAGTCC GCCTTAGGTT GGAATCATCA TTAAAGGTCA AAAGGTCGTT

+2 nLysGluGln AsnGlyValGln AspProGln HisGluArg IleIleThrVal SerThrAs
   -----

361 CAAGGAACAG AACGGAGTAC AAGATCCTCA GCATGAGAGA ATTATTACTG TGTCTACTAA
   GTTCCTTGTC TTGCCTCATG TTCTAGGAGT CGTACTCTCT TAATAATGAC ACAGATGATT

+2 nGlySerIle HisSerProArg PheProHis ThrTyrPro ArgAsnThrVal LeuValTr
   -----

421 TGGAAGTATT CACAGCCCAA GGTTCCTCA TACTTATCCA AGAAATACGG TCTTGSTATG
   ACCTTCATAA GTGTCGGGTT CCAAAGGAGT ATGAATAGGT TCTTTATGCC AGAACCATAC

+2 pArgLeuVal AlaValGluGlu AsnValTrp IleGlnLeu ThrPheAspGlu ArgPheGl
   -----

481 GAGATTAGTA GCAGTAGAGG AAAATGTATG GATACAACTT ACGTTTGATG AAAGATTG
   CTCTAATCAT CGTCATCTCC TTTTACATAC CTATGTTGAA TGCAAACCTAC TTTCTAAACC

+2 yLeuGluAsp ProGluAspAsp IleCysLys TyrAspPhe ValGluValGlu GluProSe
   -----

541 GCTTGAAGAC CCAGAAGATG ACATATGCAA GTATGATTTT GTAGAAGTTG AGGAACCCAG
   CGAACTTCTG GGTCTTCTAC TGTATACGTT CATACTAAAA CATCTTCAAC TCCTTGGGTC

+2 rAspGlyThr IleLeuGlyArg TrpCysGly SerGlyThr ValProGlyLys GlnIleSe
   -----

601 TGATGGAACT ATATTAGGGC GCTGGTGTGG TTCTGGTACT GTACCAGGAA AACAGATTTC
   ACTACCTTGA TATAATCCCC CGACCACACC AAGACCATGA CATGGTCCTT TTGTCTAAAG

+2 rLysGlyAsn GlnIleArgIle ArgPheVal SerAspGlu TyrPheProSer GluProGl
   -----

661 TAAAGGAAAT CAAATTAGGA TAAGATTGTT ATCTGATGAA TATTTTCCTT CTGAACCAGG

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Fig. 9 (cont.)

ATTTCCTTTA GTTTAATCCT ATTCTAAACA TAGACTACTT ATAAAAGGAA GACTTGGTCC

+2 yPheCysIle HisTyrAsnIle ValMetPro GlnPheThr GluAlaValSer ProSerVa

721 GTTCTGCATC CACTACAACA TTGTCATGCC ACAATTACA GAAGCTGTGA GTCCCTCAGT
CAAGACGTAG GTGATGTTGT AACAGTACGG TGTAAAGTGT CTTGCACACT CAGGAAGTCA

+2 lLeuPrcPro SerAlaLeuPro LeuAspLeu LeuAsnAsn AlaIleThrAla PheSerTh

781 GCTACCCCTT TCAGCTTTGC CACTGGACCT GCTTAATAAT GCTATAACTG CCTTTAGTAC
CGATGGGGGA AGTCGAAACG GTGACCTGGA CGAATTATTA CGATATTGAC GGAAATCATG

+2 rLeuGluAsp LeuIleArgTyr LeuGluPro GluArgTrp GlnLeuAspLeu GluAspLe

841 CTTGGAASAC CTTATTGAT ATCTTGAACC AGAGAGATGG CAGTTGGACT TAGAAGATCT
GAACCTTCTG GAATAAGCTA TAGAACTGG TCTCTCTACC GTCAACCTGA ATCTTCTAGA

+2 uTyrArgPro ThrTrpGlnLeu LeuGlyLys AlaPheVal PheGlyArgLys SerArgVa

901 ATATAGGCCA ACTTGGCAAC TTCTTGCAA GGCTTTTGT TTTGGAAGAA AATCCAGAGT
TATATCCGCT TGAACCGTTG AAGAACCCTT CCGAAAACAA AAACCTTCTT TTAGGTCTCA

+2 lValAspLeu AsnLeuLeuThr GluGluVal ArgLeuTyr SerCysThrPro ArgAsnPh

961 GGTGGATCTG AACCTTCTAA CAGAGGAGGT AAGATTATAC AGCTGCACAC CTCGTAACTT
CCACCTAGAC TTGGAAGATT GTCTCTCCA TTCTAATATG TCGACGTGTG GAGCATTGAA

+2 eSerValSer IleArgGluGlu LeuLysArg ThrAspThr IlePheTrpPro GlyCysLe

1021 CTCAGTGTC ATAAGGGAAG AACTAAAGAG AACCGATACC ATTTTCTGGC CAGGTTGTCT
GAGTCACAGG TATTCCCTTC TTGATTCTC TTGGCTATGG TAAAAGACCG GTCCAACAGA

+2 uLeuValLys ArgCysGlyGly AsnCysAla CysCysLeu HisAsnCysAsn GluCysGl

1081 CCTGGTAAA CGCTGTGGTG GGAAGTGTG CTGTTGTCTC CACAATTGCA ATGAATGTCA
GGACCAATTT GCGACACCAC CCTTGACACG GACAACAGAG GTGTTACGT TACTTACGT

+2 nCysValPro SerLysValThr LysLysTyr HisGluVal LeuGlnLeuArg ProLysTh

1141 ATGTGTCCA AGCAAAGTTA CTAATAATA CCACGAGGTC CTTCAAGTGA GACCAAAGAC
TACACAGGCT TCGTTTCAAT GATTTTTTAT GGTGCTCCAG GAAGTCAACT CTGGTTTCTG

+2 rGlyValArg GlyLeuHisLys SerLeuThr AspValAla LeuGluHisHis GluGluCy

1201 CGGTGTCAAG GGATTGCACA AATCACTCAC CGACGTGGCC CTGGAGCACC ATGAGGAGTG
GCCACAGTCC CTAACGTGT TTAGTGAGTG GCTGCACCGG GACCTCGTGG TACTCCTCAC

+2 sAspCysVal CysArgGlySer ThrGlyGly
----->

1261 TGACTGTGTG TGCAGAGGGA GCACAGGAGG ATAGCCGCAT CACCACCAGC AGCTCTTGCC
ACTGACACAC ACGTCTCCCT CGTGTCTCC TATCGGCGTA GTGGTGGTCG TCGAGAACGG

1321 CAGAGCTGTG CAGTGCAGTG GCTGATTCTA TTAGAGAACG TATGCGTTAT CTCCATCCTT
GTCTCGACAC GTCACGTCAC CGACTAAGAT AATCTCTTGC ATACGCAATA GAGGTAGGAA

1381 AATCTCAGTT GTTGCTTCA AGGACCTTTC ATCTTCAGGA TTTACAGTGC ATTCTGAAAG

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Fig. 9 (cont.)

TTAGAGTCAA CAAACGAAGT TCCTGGAAAG TAGAAGTCCT AAATGTCACG TAAGACTTTC

1441 AGGAGACATC AACAGAATT AGGAGTTGTG CAA
TCCTCTGTAG TTGTCTTAA TCCTCAACAC GTT

60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100

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Figure 10. Predicted Full-length Polypeptide Sequence

1 MSLFGLLLLT SALAGQRQGT QAESNLSSKF QFSSNKEQYG VQDPQHERII
51 TVSTNGSIHS PRFPHTYPRN TVLVWRLVAV EENVWIQLTF DERFGLEDPE
101 DDICKYDFVE VEEPSDGTIL GRWCGSGTVP GKQISKGNQI RIRFVSDEYF
151 PSEPGFCIHY NIVMPQFTEA VSPSVLPESA LPLDLLNNAI TAFSTLEDLI
201 RYLEPERWQL DLEDLYRPTW QLLGKAFVPG RKSrvVDLNL LTEEVRLYSC
251 TPRNFSVSIR EELKRTDTIF WPGCLLVKRC GGNCACCLHN CNECQCVP SK
301 VTKKYHEVLQ LRPKTGVRGL HKSLTDVALE HHEBCDCVCR GSTGG

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Figure 11 Alignment of VEGF-X with Other VEGFs

```

      20      40      60
VEGF_HUMAN : -----
PLGF_HUMAN : -----
VEGF_HUMAN : -----
VEGF_HUMAN : -----
990126vegX : XSLFGLLLLTALAGCRGQTQAESNLSSKQFSSNKQNGVQDPQHERIITVSTNGS[KSPRFPHTYP : 6

      80      100     120
VEGF_HUMAN : -----
PLGF_HUMAN : -----
VEGF_HUMAN : -----
VEGF_HUMAN : -----
990126vegX : RNTVLVWRLLVAVEZNVHILQTFDSRFGLDPSDDICKYDFVEV---EESPDDGTLGRWCSSGTVPQKCI : 13

      140     160     180     200
VEGF_HUMAN : -----
PLGF_HUMAN : -----
VEGF_HUMAN : -----
VEGF_HUMAN : ALLPSPREAPAAAAAFESGLDLSDAEPDAGZATAYASKDLEELRSVSSVDELMTVLYPEYWKHYKCC : 8
VEGF_HUMAN : ---MYREWVVVNVFMHLYVQLVQGSSEHGPVKRSGSTLEAEEQQTRAASGLLELLRITHSEDWKL : 6
990126vegX : SKGNIRIRIVSDEYFPSEFGPCIHYNIVMRFOTEAVSFVLPFSPALPLDLLNNAITAFSTLEDLIRY : 20

      220     240     260
VEGF_HUMAN : WVENSLAFLYLKHAHNSAAPHAECCGQNHHSKFFV-VG-YYNFIITLDDSCYPSIRYI : 73
PLGF_HUMAN : FPGFLQLACLAEPVPPQWALSAGNGSSSEVPFQF-VG-YYNFIITLDDSCYPSIRYI : 73
VEGF_HUMAN : SPFLRLLELAALQLAPAPVPSQDAPGHQKSW-VG-YYNFIITLDDSCYPSIRYI : 68
VEGF_HUMAN : LRKSGWQKNNREQANLSRTEETIKFAAAHYNTKSW-VG-YYNFIITLDDSCYPSIRYI : 15
VEGF_HUMAN : WRCRLRLKSTSSDERSASRSTRFAATFYDILTKVVEEIQQFVARTCTCELASLCKSTNTP : 13
990126vegX : LEPERWQECLEDPYRPTWLLGKAPVFCRKSRYDLNLTTEEVLVYCTPRNFVSIRIELKRTDTI : 27

      280     300     320     340
VEGF_HUMAN : KPSPTITND--EC--LSEEN--SR--PHQC---QH--Z--PLQ--K--E : 131
PLGF_HUMAN : SPSPTITND--EC--LSEEN--SR--PHQC---QH--Z--PLQ--K--E : 131
VEGF_HUMAN : VPSPTITND--EC--LSEEN--SR--PHQC---QH--Z--PLQ--K--E : 131
VEGF_HUMAN : KPSPTITND--EC--LSEEN--SR--PHQC---QH--Z--PLQ--K--E : 211
VEGF_HUMAN : KPSPTITND--EC--LSEEN--SR--PHQC---QH--Z--PLQ--K--E : 191
990126vegX : WPSPTITND--EC--LSEEN--SR--PHQC---QH--Z--PLQ--K--E : 331

      360     380     400
VEGF_HUMAN : KNDPRARZEXKSVAGKCKQKRRKXSRYSKMSVP- : 141
PLGF_HUMAN : KNDPRARZEXKSVAGKCKQKRRKXSRYSKMSVP- : 141
VEGF_HUMAN : KNDPRARZEXKSVAGKCKQKRRKXSRYSKMSVP- : 139
VEGF_HUMAN : KNDPRARZEXKSVAGKCKQKRRKXSRYSKMSVP- : 281
VEGF_HUMAN : KNDPRARZEXKSVAGKCKQKRRKXSRYSKMSVP- : 241
990126vegX : KNDPRARZEXKSVAGKCKQKRRKXSRYSKMSVP- : 341

      420     440     460
VEGF_HUMAN : -----
PLGF_HUMAN : -----
VEGF_HUMAN : -----
VEGF_HUMAN : GPVKELDSEETCCQCVCRAGLAPASCCGPHKELDANSQCQCVCNNKLFPSQCGANREFDENTCCQCVCRKTC : 348
VEGF_HUMAN : -----
990126vegX : ----- : 286

      480     500     520     540
VEGF_HUMAN : TCKS-SCKNTDSR--KARQLSLNERTCRCDKPR- : 215
PLGF_HUMAN : TCKS-SCKNTDSR--KARQLSLNERTCRCDKPR- : 215
VEGF_HUMAN : TCKS-SCKNTDSR--KARQLSLNERTCRCDKPR- : 186
VEGF_HUMAN : TCKS-SCKNTDSR--KARQLSLNERTCRCDKPR- : 416
VEGF_HUMAN : TCKS-SCKNTDSR--KARQLSLNERTCRCDKPR- : 354
990126vegX : TCKS-SCKNTDSR--KARQLSLNERTCRCDKPR- : 354

VEGF_HUMAN : ----- : -
PLGF_HUMAN : ----- : -
VEGF_HUMAN : ----- : -
VEGF_HUMAN : CHS----- : 419
VEGF_HUMAN : ----- : -
990126vegX : ----- : -

```

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Figure 13. Primers for Expression of VEGF-X

E.coli expression of domain-

vegX-6 AATTGGATCCGAGAGTGGTGGATCTGAACC
vegX-7 AATTGGATCCGGAAGAAAATCCAGAGTGG
vegX-8 GGTTGAATTCATTATTTTTAGTAACTTTGCTTGGGACAC
vegX-9 AATTGAATTCATTATCCTCCTGTGCTCCCTC

Baculovirus/insect cell expression of full-length protein-

vegbac1
AATTGGATCCGGAGTCTCACCATCACCACCATCATGAATCCAACCTGAGTAGTAAATTC
C
vegbac2 AATTGAATTCGCTATCCTCCTGTGCTCCCTCTGC

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1 >3993180H1 LUNGNON03 INCYTE
2 CACAAATCACTCACCGACGTGGCCCTGGAGCACCATGAGGNGTGTGACTGTGTGTGCAGAGGGAGCACAGGAGGATAGCC
3 GCATCACCACCAGCAGCTCTTGGCCAGAGCTGTGCAGTGCAGTGGCTGATTCTATTAGAGAACGTATGCGTTATCTCCAT
4 CCTTAATCTCAGTTGTTTGGCTTCAAGGACCTTTCATCTTCAGGATTTACAGTGCATTCTGAAAGAGGAGACATCAAACAG
5 AATTAGGAGTTGTGCAACAGCTCTTTGAGAGGAGGCTAAAGACAGGAGAAAGGCTT
6 >3510192H1 CONCN01 INCYTE
7 TGCAGTGCAGTGGCTGATTCTATTAGAGAACGTATGCGTTATCTCCATCCTTAATCTCAGTTGTTTGGCTTCAAGGACCTT
8 TCATCTTCAGGATTTACAGTGCATTCTGAAAGAGGAGACATCAAACAGAAATTAGGAGTTGTGCAACAGCTCTTTTGAGAG
9 GAGCCCTAAAGGACAGGAGAAAGSTCTTCAATCGTGGAAAGAAATTAATGTTGTATTAAATAGATCACCAGCTAGTT
10 TCAGAGTTACCATGTACGTATTCACCTAGCTGGGTTCTGTATTT
11 >2559870H1 ADRETUT01 INCYTE
12 CAGGAGGTCCTTCAGTTGAGACCAAGACCGGTGTCAGGGGATTGCACAAATCACTCACCGACGTGGCCCTGGAGCACCA
13 TGAGGAGTGTGACTGTGTGTCGAGGGAGCACAGGGGATAGCCGCATCACCACCAGCAGCTCTTGGCCAGAGCTGTGTC
14 AGTCCAGTGGCTGATTCTATTAGAGAACGTATGCGTTATCTCCATCCTTAATCTCAGTTGTTTGGCTTCAAGGACCTTTCA
15 TCTTCAGGATTTACAGTGCATTCTGAAAGAGGAGA
16 >3979767H1 LUNGUT08 INCYTE
17 GGAGGATAGCCGCATCACCACCAGCAGCTCTTGGCCAGAGCTGTGCAGTGCAGTGGCTGATTCTATTAGAGAACGTATGC
18 GTTATCTCCATCCTTAATCTCAGTTGTTTGGCTTCAAGGACCTTTTCATCTTCAGGATTTACAGTGCATTCTGAAAGAGGAG
19 ACATCAAACAGAAATTAGGAGTTGTGCAACAGCTCTTTTGAGAGGAGGCCCTAAAGGACAGGAGAAAGGCTCTTCAATCGTG
20 GAAAGAAATTAATGTTCTATTAAATAGACACCACT
21 >3980011H1 LUNGUT08 INCYTE
22 GGAGGATAGCCGCATCACCACCAGCAGCTCTTGGCCAGAGCTGTGCAGTGCAGTGGCTGATTCTATTAGAGAACGTATGC
23 GTTATCTCCATCCTTAATCTCAGTTGTTTGGCTTCAAGGACCTTTTCATCTTCAGGATTTACATGCATTCTGAAAGAGGAGA
24 CATCAAACAGAAATTAGGAGTTGTGCAACAGCTCTTTTGAGAGGAGGCCCTAAAGGACAGGAGAAAGGCTCTTCAATCGTG
25 AAAGAAATTAATGTTCTATTAAATAGATCACC
26 >4825396H1 BLADDIT01 INCYTE
27 GAGAACCCGATACCATTTCTGGCCAGGTTGTCTCTGTTAAACCGCTGTGGTGGGAACCTGTGCCTGTGTCTCCACAATT
28 GCAATGAAATGTCAATGTGTCACCAAGCAAGTTACTAAAGAAATACCACAGGTCCTTCAGTTGAGACCAAGACCGGTGTC
29 AGCGGATTCACAAATCACTCACCGAGCTGGCCCTCGAGCACCATGAGGAGTGCTACTGTGTGTCAGAGGGAGCACAGC
30 AGGATAGCCGCATCACCACCA
31 >3073703H1 BONEUNT01 INCYTE
32 AGAAATCCAGAGTGGTGGATCTGAACCTTCTTAACAGAGGAGTAAGATTATACAGCTGCACACCTCGTAACCTTCTCAGT
33 GTCCATAAGGGAAGAACTAAAGAGAACCGATACCATTTTCTGGCCAGGTTGTCTCTGTTAAACCGCTGTGGTGGGAAC
34 GTGCCTGTGTGTCTCCACAATTGCAATGAATGTCAATGTGTCACCAAGCAAGTTACTAAAAAATACCACGAGGTCCTTCAG
35 TTGAGACCAAGACCGGTGTACGGGATTGACACAAATCA
36 >1302516H1 PLACNOT02 INCYTE
37 AGGAATCAAATTAGGATAAGATTGTATCTGATGAATATTTTCTCTTGAACCTTCTAACAGAGGAGGTAAGATTATAC
38 AGCTGCACACCTCGTAACCTTCTCAGTGTCCATTAAGGGAAGAACTAAAGAGAACCGATACCATTTTCTGGCCAGGTTGTCT
39 CCTGGTTAAACGCTGTGGTGGGAACCTGTGCTCTCCACAATTGCAATGAATGTCAATGTGTGTCACCAAGCAAGTT
40 ACTAAAAAATACCACGAGGTC
41 >3684109H1 HZANCT01 INCYTE
42 ATTTTCATCTTCAGGATTTACAGTGCATTCTGAAANAGGAGAAATCAAACANAATTAGGAGTTGTGCAACAGCTCTTTTGA
43 GAGGAGGCTTAAAGCACAGGAGAAAGGCTTCAATCGTGGGAAANAAATTAATGTTGTATTAAATAGATCACCAGCTA
44 GTTTCAGAGTTACCATGTACGTATTCACCTAGCTGGGTTCTGTATTTTCAGTTCTTTCGATACGGCTTAGGGTAATGTGTCAG
45 TACAGCAAAAAAATGTGCAAGTGAACCTGATTCGGTTGCCCTTGCT
46 >4713188H1 BRAINCT01 INCYTE
47 CAAAGTCTACTAAAAAATACCACCAAGTCTTCAGTTGACACCAAGACCGGTGTGAGGGGATTGCACAAATCACTCACCG
48 ACGTGGCCCTGGAGCACCATGAGCAGTGTGACTGTGTGTGTCAGAGGGAGCACAGGAGGATAGCCGCATCACCACCAGCAG
49 CTCTTGGCCAGAGCTGTGCACTGCAGTGGCTGATTCTATTAGAGAACGTATGCGTTATCTCCATCCTTAATCTCAGTTGT
50 TTGCT
51 >458823H1 XERANOT01 INCYTE
52 ANGAGTTGCCCCAGAGCTGTGCACTGCAGTGGCTGATTCTATTAGAGAACGTATGCGTTATCTCCATCCTTAATCTCAGTT
53 GTTGTGTTCAAGGACCTTTCATCTTCAGGATTTACAGTGCATTCTGAAAGAGGAGACATCAAACAGAAATTAGGAGTTGTG
54 CAACACCTCTTTTGAGAGGAGGCCCTAAAGCNCAGGACAAAAGGCTTCAATCGTGGAAAGAAATTAATGTTGTATTAA
55 ATAGATC
56 >1303909H1 PLACNOT02 INCYTE
57 AGGAATCAAATTAGGATAAGATTGTTGTATCTGATGAATATTTCTCTTGAACCTTCTAACAGAGGAGGTAAGATTATAC
58 AGCTGCACACCTCGTAACCTTCTCAGTGTCCATAAGGGAAGAACTAAAGAGAACCGATACCATTTTCTGGCCAGGTTGTCT
59 CCGGTTAAAGCCTGTGGTGGGAACCTGTGCTGTTGTCTCCACAATTGCAATGAATGTCAATGTGTGTCACCAAG
60 >2739211H1 OVARNOT09 INCYTE
61 GTGCATTCTGAAAGAGGAGACATCAACAGAAATTAGGAGTTGTGCAACAGCTCTTTTGAGAGGAGGCCCTAAAGGACAGGA
62 GAAAGGCTTCAATCGTGGAAAGAAATTAATGTTGTATTAAATAGATCACCAGCTAGTTTCAGAGTTACCATGTACG
63 TATTCACCTAGCTGGGTTCTGTATTTTCAGTTCTTTTCGATACGGCTTAGGGTAATGTGATACAGGAAAAAATCTGTGCAA
64 GTGAGCAGCTGAT
65 >3325591H1 PTHYN01 INCYTE
66 TGCAACAGCTCTTTTGACAGCAGGCCCTAAAGGACAGGAGAAACCTCTTCAATCGTGGAAAGAAATTAATGTTCTATT
67 AAATAGATCACCAGCTAGTTTCAGAGTTACCATGTACGTATTCACCTAGCTGGGTTCTGTATTTTCAGTTCTTTTCGATACG
68 GCTTAGGGTAATGTGATACAGGAAAAAATCTGTGCAAGTGAGCAGCTGATTCGGTTGCCCTTGCTTAACCTTAAGCNC
69 ATGTCNNGGGCNAARANCAGAAAAAT
70 >3733565H1 SMCCN0501 INCYTE
71 CCTTAATCTCAGTTGTTTGGCTTCAAGGACCTTTCATCTTCAGGATTTACAGTGCATTCTGNAAGANGAGACATCAAACAG
72 AATTAGGNGTTGTGCAAAAGCTCTTTTGAGAGGAGGCCCTAAAGGACAGGAGAAACCTCTNCAATCGTGGAAAGNAAT
73 AAATGTTGATNAATNGATCACCAGCTAGTTTCAGAGTTACCATGTACGTATTCACCTAGCTGGGNCNGTATTACGTCT
74 TTCGAAACGGCTTAGGGTAATGTGATACAGGAAAAAATCTGTGAGTGAG
75 >3554223H1 SYNONOT01 INCYTE

Fig. 14

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Fig 14 (cont'd)

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76 >877279H1 LUNGAST01 INCYTE
77 CTTTTTATGACAACTTAGATCAACTATTTTACGCTTGGTAAATTTTCTAAACACAATTGTTATAGCCAGAGGAACAAA
78 GATGATATAAAAATATTTGCTCTGACAAAAATACATGATTTTCAATCTCTGATGGTCTAGAGTTAGATTAACTGTCAT
79 TTTAAAAAACTCAATTGGAAATAGAAATGGTAAAGTTGCAAGGCTTTTGAATAAATAAATTATCATATCTTCCATTCC
80 TGTATTGGNGG
81 >4713188H1 BRAINKT01 INCYTE
82 CAAAGCTACTAAAAAATACCACGAGTCTTCAAGTTGACACCAAGACCGGTGTCAGGGGATTCCACAAATCACTCACCC
83 ACGTGGCCCTGGAGCACCATGAGGAGTGTGACTGTGTGTCAGAGGGAGCAGGAGGATAGCCGCATCACCACCAGCAG
84 CTCTTGGCCAGAGCTGTGCACTGCACTGCTGATTCTATTAGAGAACGATGCGTTATCTCCATCCTTAATCTCAGTTGT
85 TTGCT
86 >2171082H1 ENDCNOT03 INCYTE
87 AGATAAACCTGAAAAGAGAGTGGCCTTATCTTCACTTTATCGATAAGTCAGTTTATTTGTTTCATTGTGTACATTTT
88 TATTTCTCTTTTGACATTATAACTGTGGCTTTTCTAATCTTGTAAATATATCTATTTTACCAGAGGTATTTAATATT
89 CTTTTTATGACAACTTAGATCAACTATTTTAGCTTGGTAAATTTTCTAAACACAATTGTTATAGCCAGAGGAACAAA
90 GATGA
91 >875860H1 LUNGAST01 INCYTE
92 CTGGATTTTTCATATTTCTTATTAATAATTTCTGCCATTTAGAAGAAGAGAACTACATTCATGGTTTGGAGAGATAAACCC
93 TGAAAAGAGAGAGTGGCCTTATCTTCACTTTATCGATAAGTCAGTTTATTTGTTTCATTGTGTACATTTTATATTCTCT
94 TTTGACATTATAACTGTTGGCTTTCTAATCTTGTAAATATATCTATTTTACCAGAGGTATTTAATATTCTTTTAT
95 GAC
96 >706168H1 SYNORAT04 INCYTE
97 GCTCATATTCACATATGTAAACCAGAACATTCTATGTACTACAAACCTGGTTTTTAAAAAGGANCATGTTGCTATGAAT
98 TAAACTTGTGTCGTGCTGATAGACAGACTGGATTTTTCATATTTCTTATTAATAATTTCTGCCATTTAGAAGAAGAGAAC
99 TACATTCATGGTTTGGAGAGATAAACCTGAAAAGAGAGTGGCCTTATCTTCAATTTATCGATAAGTCAGTTTATTTGT
100 TTCA
101 >459223H1 KERANOT01 INCYTE
102 ANGAGTTGCCAGAGCTGTSCASTGCGAGTGGCTGATTCTATTAGAGAACGATGCGTTATCTCCATCCTTAATCTCAGTT
103 GTTTGNTTCAAGGACCTTTTCATCTTCAGGATTTACAGTGCATTCTGAAAAGAGGAGACATCAAAACAGAAATAGGAGTTGTG
104 CAACAGCTCTTTTGACAGGAGGCCATAAGGNCAGGAGAAAAAGCTCTTCAATCGTGGAAAAGAAAATTAATGTTGTATTAA
105 ATAGATC
106 >538436H1 LNCN0T02 INCYTE
107 AAAGATGATATAAAATATGTTGCTCTGACAAAAATACATGATTTTCATTCTCGTATGGTCTAGAGTTAGATTAACTCTG
108 CATTTTAAAAAACTGAATTCGAATAGAAATGGTAAAGTTCAGAACTTTTGAATAATTAATTAATTCATATCTTCCAT
109 CTCTGTTTAAAGCTGAGATGAAAATAAAAAGCAATTTATGAAGTAGACATTCAGATCCAGCCATTACTAACCCTAT
110 >1303909H1 FLACNOT02 INCYTE
111 AGGAAATCAAAATAGGATAAGATTGTATCTGATGAATATTTCTCTTCTGAACCTTCTAACAGAGAGGGTAAGATTATAC
112 AGCTGCACACCTCGTAACCTCTCAGTCTCCATAAGGGAAGAACTAAGAGAAACGGATACCATTTCTGGCCAGGTTGTCT
113 CCTGTTTAAAGCTGTGGTGGAACTGTCGCTGTTGTCTCCCAATTTGCAATGAATGTCATGTTGTGCCAAG
114 >2739211H1 OVARNOT09 INCYTE
115 GTCCATTCTGAAGAGGAGACATCAAAACAGAAATAGGAGTGTGTGCAACAGCTCTTTTGAAGAGGAGCCATAAGGACAGGA
116 GAAAAGGTCTTCAATCGTGGAAAGAAAATTAATGTTGTATTAATAGATCACCAGCTAGTTTCAGAGTTACCATGTACG
117 TATTCACATAGCTGGGTTCTGTATTTCAGTTCTTTGATACGGCTTAGGGTAATGTCTAGTACAGGAAAAAACTGTGCAA
118 GTGAGCACCTGAT
119 >2550343H1 LUNGUT06 INCYTE
120 TGTACATTTTATATTCTCTTTTGACATTATAACTGTTGGCTTTTCAATCTTGTAAATATATCTATTTTACCAGG
121 GTATTTAATATCTTTTATGACAACTTAGATCAACTATTTTACCTGCTAAATTTTCTAAACACAATTGTTATAGC
122 CAGAGGAACAAAGATGATATAAAATATGTTGCTCTGACAAAAATACATGATTTTCAATCTCTGATGGTGCTA
123 >5321148H1 FIBPFEN:6 INCYTE
124 CACAATTTGTTATAGCCAGAGGAACAAAGATGATATAAATATTTGCTCTGNCAAAAATACATGATTTTCATTCTCGTA
125 TGGTCTAGAGTTAGATTAACTCTCAATTTAAAAAACTGAAATGGAATAGAATTGGTAAGTTGCAAAAGACTTTTGA
126 TAATTAATTTATCATATCTTCCATCTCTTATTGGAGATGAAAATAAAAGCAACTTATGAAAGTAAATTCAGATCCAC
127 CATTACTAAC
128 >979495H1 THYRNOT02 INCYTE
129 ATTTCAATCTCGTATGGTCTAGAGCTTAGATTAACTGCAATTTTAAATACTGAAATGGAATAGAATTGGTAAGTTGCAA
130 AGACTTTTGAATAATTAATTAATATCTTCCATCTCTGTTATTGGAGATGAAAATAAAAGCAACTTATGAAAGT
131 AGACATTCAGATCCAGCCATTACTACCTATTCCTTTTGGGAAATCTGAGCTAGCTCAGAAAAACATAAAGCACCT
132 TGAAAAA
133 >3325591H1 PTHYNCT03 INCYTE
134 TGCAACAGCTCTTTTGAGAGGAGGCTTAAAGGACAGGAGAAAAGGCTCTCAATCGTGGAAAAGAAAATTAATGTTGTATT
135 AAATAGATCACCAGCTAGTTTCAGAGTTACCATGTACGTATTCACCTAGCTGGGTCTGTATTCAGTTCTTTCGATACG
136 CCTTAGGCTAATGTCTAGTACAGGAAAAAAGCTGTGCAAGTGAGCACCTGATTCGGTTGCTTACCCCTAAAGCNC
137 ATGTCNNGGCNAANCGAAAAAT
138 >543890H1 OVARNOT02 INCYTE
139 TTTCTAAACACAATTGTTATAGCCAGAGGAACAAAGATGATATAAAATATTTGCTCTGACAAAAATACATGTAATTCA
140 TTCTCGTATGGTCTAGAGTTAGATTAACTCTGCAATTTTAAAAAACTGAAATGGNATAGAAATGGTAAGTTGCAAGNCTT
141 TTTGAAAATAATTAATTAATTAATCTTCCATTCCTGTTATTGGAGGATGGAATAAATAAGCAACTTATGGAAAGTAGG
142 ACATTACAGATC
143 >3732565H1 SMCCNOS:1 INCYTE
144 CCTAATCTCAGTTGTTTGTCTCAAGGACCTTTCACTTTCAGGATTACAGTGCATTCTGNAAGANGAGACATCAAAACAG
145 AATTAGGNSTTGTGCAAAAGCTCTTTTGAGAGGAGGCTTAAAGGACAGGAGAAAAGGCTCTNCAATCGTGGAAAGNAAAT
146 AAAGCTGTATTAATAATGATCACCAGCTAGTTTCAGAGTTACCATGTACGTATTCACCTAGCTGGGNCNGTATTCAGTCT
147 TCCGGAACCCCTTAGCGTAATGTCTAGTACAGGAAAAAACTGTGCAAGTGAG
148 >4641939H1 PROSTMTC3 INCYTE
149 GTACTACAAACCTGGTTTTTAAAGGPACTATGTTGCTATGAATTAAGTTGTTGCTCATGCTGATAGGACAGACTGGAT
150 TTTTCATATTTCTTATTAATAATTTCTGCCATTTAGAAGAGAGAACTACATTCATGGTTTGSNAGAGATAAACCTGAAAA

Fig. 15 (cont.)

[illegible]

Express Mail Label No.

226 CTTTTAAAAAC TGANTTGGATAGANTTGGTAAGTTGCAAAGNCNTTTGAAAAATNATTAAQTATCAGAT
 227 >3530274H1 BLADNOT09 INCYTE
 228 TTCCATTCCCTGTTATTGGAGATGAAAAATAAAAAGCAACTTATGAAAGTAGACATTTCAGATCCAGCCATTACTAACCTATT
 229 CCTTTTTTGGGGAAATCTGAGCCTAGCTCAGAAAAACATAAAGCACCTTGAAAAAGACTTGGCAGCTTCCTGATAAAGCG
 230 TGCTGTGCTGTGCAQTAGGAACACATCCTATTTATGTGTGATGTGTGCTTTATTATCTAAACTCTGTTCATACACTTG
 231 TATAAATACATGGATATTTTATGTACAGAAATATCTCTTAAACAGTTCA
 232 >3530249H1 BLADNOT09 INCYTE
 233 CTTCATTCCCTGTTATTGGAGATGAAAAATAAAAAGCAACTTATGAAAGTAGACATTTCAGATCCAGCCATTACTAACCTAT
 234 TCCTTTTTTGGGGAAATCTGAGCCTAGCTCAGAAAAACATAAAGCACCTTGAAAAAGACTTGGCAGCTTCCTGATAAAGCG
 235 GTGCTGTGCTGTGCAQTAGGAACACATCCTATTTATGTGTGATGTGTGCTTTATTATCTTAAACTCTGTTCATACACT
 236 TGTATAAATACATGGATATTTTATGTACAGAAATATGTCTCTTAAACAGTTCACTTATTCTACCTGG
 237

Fig 15 (cont'd)

05463647 422499

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VEGFE1	AAAATGTATGGATACAACTTAC	22
VEGFE2	GTTTGATGAAAGATTGCGCTTG	23
VEGFE3	TTTCTAAAGGAAATCAAATTAG	22
VEGFE4	GATAAGATTTGTATCTGATG	20
VEGFE5	GATGTCTCCTCTTTCAG	17
VEGFE6	GCACAACTCCTAATTCTG	18
VEGFE7	AGCACCTGATTCCGTTGC	19
VEGFE8	TAGTACATAGAATGTTCTGG	20
VEGFE9	AAGAGACATACTTCTGTAC	19
VEGFE10	CCAGGTACAATAAGTGAAGT	21

Fig. 16

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EL024661737US
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0546644 423450

```
+3      M N I F L L N L L T E E V R L Y
1  AGGAAATCAA ATTAGGATAA GATTGTATC TGATGAATAT TTTCCTTCTG AACCTTCTAA CAGAGGAGGT AACATTATAC
   TCCTTTAGTT TAATCCTATT CTAACATAG ACTACTTATA AAAGGAACAC TTGGAAGATT GTCTCTCCA TTCTAATATG
.....
+3  S C T P R N P S V S I R E E L K R T D T I F W P G C I
81  AGCTGCACAC CTCGTAACTT CTCAGTGTCC ATAAGGGAAG AACTAAAGAG AACCGATACC ATTTCTGTGC CAGGTTGTCT
   TCGACGTCTG GAGCATTGAA GAGTCACAGG TATTCCTTC TTGATTTCTC TTGCTATGG TAAAGACCG GTCCAACAGA
-2
.....
+3  L V K R C G G N C A C C L H N C N E C Q C V P S K V
161  CCTGGTTAAA CGCTGTGGTG GGAACGTGTC CTGTGTCTC CACAATTCCA ATGAATGTCA ATGTGTCCA ACCTAAGTTA
   GGACCAATTT CGGACACAC CTTTGACAGG CACAACACAG GTGTTAACGT TACTTACAGT TACACAGGT TCGTTTCAAT
-2
.....
+3  T K K Y K E V L Q L R P X T G V R G L K K S L T D V A
+1      V S G D C T N H S P T W P
241  CTAAAAATA CCACGAGGTC CTTCACTTGA GACCAAGAC CGGTGTCAAG GGATTGCACA AATCACTCAC CGACGTGGCC
   GATTTTAT GGTGCTCCAG GAAGTCAACT CTGTTTCTG CCGACAGTCC CTAACGTGT TTAGTGAGTG GCTGCACCG
-2
.....
+3  L E H H E E C D C V C R G S T G G
+2      V Q P E H R R I A A S P P A A L A
+1  W S T M R S V T V C A Z G A Q E D S R I T T S S S C
321  CTGGAGCACC ATGAGGAGTG TGAAGTGTG TGCAGAGGA GCACAGGAGG ATAGCCGCAT CACCAACAGC AGCTCTTCC
   GACCTCGTG TACTCTCAC ACTGCACAC AGCTCTCCCT CGTGTCTTC TATCGCGTA GTGTGGTGC TGCAGACCG
.....
+2  Q S C A V Q W L I L L E N V C V I S I L N L S C L L C
+1  P E L C S A V A D S I R E R M R Y L H P
401  CAGAGCTGTG CAGTGCAGTG GCTGATTCTA TTAGAGAAG TATGCGTTAT CTCCATCCTT AATCTCAGTT GTTTCCTTCA
   GTCTCGACAC GTCACGTAC CCACTAAGAT AATCTCTTC ATACCGAATA GAGGTAGGAA TTAGAGTCAA CAAACGAGT
.....
+2  G P F I F R I Y S A F
481  AGACCTTC ATCTTCAGGA TTACAGTGC ATTCTGAAG AGGAGACATC AACAGAATT AGGAGTTCTG CACAGCTCT
   TCGTGAAG TAGAGTCTT AATGTCAGG TAAGACTTC TCTCTGTAG TTTGTCTTAA TCTCAACAC GTGTGAGA
.....
561  TTTGAGAGGA GGCCTAAGG ACAGGAGAAA AGGTCTTCAA TGTGGGAAAG AAAATTAAAT GTGTATTAA ATAGATCAC
   AAACCTCTCT CCGGATTTCC TGTCTCTTT TCCAGAAGT AGCACCTTC TTTTAATTA CACATAATT TATCTAGTG
.....
641  AGCTAGTTT AGAGTTACCA TGTACGTATT CCACTAGCTG GGTCTGTAT TTCAGTTCTT TCGATACCC TTAGGGTAAT
   TCGATCAAG TCTCAATGGT ACATGCATAA GGTGATCGAC CCAAGACATA AAGTCAAGAA AGCTATGCCG AATCCATTA
.....
721  GTCAGTACAG GAAAAAACT GTCAAGTGA GCACCTGATT CCGTTCCTT GGCTTAATC TAAAGCTCCA TGTCTGGCC
   CAGTCATGTC CTTTTTTTGA CAGTTCACT CGTGGACTAA GGCAACGAA CCGAATTGAG ATTTGAGGT ACAGGACCG
.....
801  CTAAAAATCT ATAAAAATCT GA
   GATTTAGCA TATTTAGAC CT
```

Fig 17

09463647 "422499

```

+3      M N I F L L N L L T E E V ~ R L Y
1  AGGAAATCAA ATTAGGATAA GATTGTATC TGAATGAAT TTTCTTCTG AACCTTCTAA CAGAGGAGGT AAGATTATAC
   TCCTTTAGTT TAATCTTATT CTAAACATAG ACTACTTATA AAAGGAAGAC TTGGAACATT GTCTCTCCA TTCTAATATG
+3  S C T P R N P S V S I R E E L K R T D T I F W P G C L
81  AGCTGCACAC CTCGTAACTT CTCAGTGTCC ATAAGGGAAG AACTAAAGAG AACCGATACC ATTTTCTGCC CAGGTGTCT
   TCGACGTGTG GAGCATTGAA GAGTCACAGG TATTCCTTC TTGATTCTC TTGGCTATGG TAAAGACCG GTCCACAGA
-2
+3  L V K R C G G N C A C C L H N C N E C Q C V P S K V
161 CCTGGTTAAA CGCTGTGGTG GGAAGTGTG CTGTGTCTC CACAATTGCA ATGAATGCA ATGTGTCCA AGCAAGTTA
   GGACCAATT GCGACACCAC CTTTACACG GACACAGAG GTGTAACTT TACTTCACT TACACAGGT TCGTTTCAAT
-2
+3  T K K Y H E V L Q L R P K T G V R G L H K S L T D V A
+1      V S G D C T N H S P T W P
241 CTAAAAATA CCAGGAGTC CTTCACTTGA GACCAAGAC CGGTGTGAGG GGATTGCACA AATCACTCAC CGAGGTGCC
   GATTTTAT GGTGTCTCAG CAAGTCACT CTGTTTCTG GCCACAGTC CCTAAGCTGT TTAGTGAGT GCTGCACCG
-2
+3  L E H H E E C D C V C R G S T G G
+2      V Q R E H R R T A A S P P A A L A
+1  W S T H R S V T V C A E G A Q E D S R I T T S S S C
321 CTGGAGCACC ATGAGGAGTG TGAAGTGTG TGCAGAGGGA GCACAGGAGG ATAGCCGCA CACCACCAGC AGCTCTTGC
   GACCTCGTGG TACTCTCAC ACTEACACAC AGGTCTCCTT CCGTCTCTCC TATCGGCGTA GTGGTGGTGG TCGAGAAGCG
-2  Q S C A V Q W L I L L E N V C V I S I L N L S C L L Q
+1  P B L C S A V A D S I R E R M R Y L H P
401 CAGAGCTGTG CAGTGCAGTG CCGATTCTA TTAGAGAAGG TATGCTTAT CTCCATCCTT AATCTCAGTT GTTGGCTCA
   GTCTCGACAC GTCACGTCAC CGACTAAGAT AATCTCTGC ATACGCAATA GAGGTAGGAA TTAGAGTCAA CAAACGAAGT
-2  G P F I F R I Y S A F
481 AGGACCTTTC ATCTTCAGGA TTTACAGTGC ATCTCGAAAG AGGAGACATC AAACAGAATT AGGAGTGTG CAACAGCTCT
   TCCTCGAAAG TAGAAGTCTT AATGTACAG TAAGACTTTC TCCTCTGTAG TTTGTCTTAA TCCTCAACAC GTTGTCCAGA
561 TTTGAGAGGA GGCCTAAAGG ACACGAGAAA AGGTCTTCAA TCGTGGAAAG AAAATTAAAT GTTGTATTAA ATAGATCACC
   AAACCTCTCT CCGGATTTC TGTCTCTTT TCCAGAGTT AGCACCTTTC TTTTAATTAA CAACATAATT TATCTAGTGG
641 AGCTAGTTTC AGAGTTACCA TGTACGTATT CCACTAGCTG GTTCTGTAT TTCAGTCTT TCGATACGGC TTACGGTAAT
   TCGATCAAG TCTCAATGCT ACATGCATAA GGTGATCGAC CCAAGACATA AAGTCAAGAA AGCTATGCCG AATCCCATTA
721 GTCAGTACAC GAAAAAACT GTGCAAGTGA GCACCTGATT CCGTTCCTT GGTTAATCT TAAAGCTCCA TGTCTGGGC
   CAGTCAATGTC CTTTMTTGA CAGCTTCACT COTGGACTAA GGCACCGAA CCGAATTGAG ATTTGAGGT ACAGGACCCG
801 CTAAAACTGT ATAAATCTG GATTTTNTN TTTTMTTGG CGCATATCA CATATGTAAA CCAGAACATT CTATGTACTA
   GATTTTAGCA TATTTTAGAC CTAAAAAAN AAAAAAAGC GCGTATAAGT GTATACATTT GTCTTGTAA GATACATGAT
881 CAAACCTGGT TTTTAAAAAG GAATATGTT GCTATGAAT AAACCTGTGT COTGCTGATA GCACAGACTG GATTTTTCAT
   GTTGGACCA AAAATTTTC GTGATACAA CGAATCTAA TTTGAACACA GCAGACTAT COTGCTGAC CTAAAAAGTA
-3

```

Fig 18

961 ATTCTTATT AAAATTCTG CCATTAGAA GAAGAGAACT ACATTCATGG TTTGGAAGAG ATAAACCTGA AAGAGAGAGT
 TAAAGATATA TTTAAAGAC GCTAAATCTT CTTCTCTTGA TGTAACTACC AAACCTTCTC TATTTGGACT TTCTTCTCA
 -3 -----

 1041 GGCCTTATCT TCACTTTATC GATAAGTCAG TTTATTTGTT TCATTCTGTA CATTTTTATA TTCTCCTTTT GACATTATAA
 CCGCAATAGA AGTGAATAG CTATTCAGTC AAATAAACAA ACTAACACAT GTAAAAATAT AAGAGGAAAA CTGTAATATT
 -3 -----

 1121 CTGTGGCTT TTCTAATCTT GTTAAATATA TCTATTTTAA CCAAAGGTAT TTAATATTCT TTTTATGAC AACTTAGATC
 GACAACCGAA AAGATTAGAA CAATTTATAT AGATAAAAT COTTTCCATA AATTATAAGA AAAAATACTG TTGAATCTAG

 1201 AACTATTTT AGCTTGGTAA ATTTTCTTAA ACACAATTC TATAGCCAGA GGAACAAAGA TGATATAAAA TATTGTGCT
 TTGATAAAAA TCGAACCATT TAAAAAGATT TGTGTTAACA ATATCGGTCT CTTGTGTTCT ACTATATTTT ATAACAACGA

 1281 CTGACAAAA TACATGTATT TCATTCTCGT ATGGTGCTAG AGTTAGATTA ATCTGCATTT TAAAAACTG AATTGGAATA
 GACTGTTTT ATGTACATAA AGTAAGAGCA TACCACGATC TCAATCTAAT TAGACGTAAA ATTTTTTGAC TTAACCTTAT

 1361 GAATTGGTAA GTTGCAAGA CTTTTTGAAG ATAATTAAAT TATCATATCT TCCATTCTCT TTAITGGAGA TGAATAATAA
 CTTAACCATT CAACGTTTCT GAAAAACTTT TATTAATTAA ATAGTATAGA AGGTAGGAC AATAACCTCT ACTTTTATT

 1441 AAGCAACTTA TGAAGTAGA CATTGAGATC CAGCCATTAC TAACCTATTC CTTTTTGGG GAAATCTGAG CCTAGCTCAG
 TTCGTGAAT ACTTTCATCT GTAACTCTAG CTCGGTAATG ATTGCATAAG GAAAAAACC CTTTAGACTC GGATCGAGTC

 1521 AAAACATAA AGCACCCTGA AAAAGACTTG GCAGCTTCTT GATAAGCGT GCTGTGCTGT GCAGTAGGAA CACATCCTAT
 TTTTGTATT TCGTGAAGT TTTCTGAAC CGTGAAGGA CTATTTCGCA CGACAGACA CGTATCTCTT GTGTAGGATA

 1601 TTATTGTGAT GTTGTGGTT TATTATCTTA AACTCTGTC CATACACTTG TATAATACA TGGATATTT TATGTACAGA
 AATAACACTA CAACACCAA ATAATAGAAT TTGAGACAAG GTATGTGAAC ATATTTAATG ACCTATAAAA ATACATGCT

 1681 AGTATGCTC TTAACCAGT CACTATTGT ACCTGG
 TCATACAGAG AATTGCTCA GTGAATAACA TGGACC

Fig 18 (cont'd)

Figure 19. DNA and polypeptide sequence used for mammalian cell expression

```

+1      m s l f g l l l l c s a l a g q r
1  GGATCCAAAA TGAGCCTCTT CGGGCTTCTC CTGCTGACAT CTGCCCTGGC CGGCCAGAGA

+1  q g t q a e s n l s s k f q f s s n k e
61  CAGGGGACTC AGGCGGAATC CAACCTGAGT AGTAAATTC AGTTTCCAG CAACAAGGAA

+1  Q N G V Q D P Q H E R I I T V S T N G S
121 CAGAACGGAG TACAAGATCC TCAGCATGAG AGAATTATTA CTGTGTCTAC TAATGGAAGT

+1  I H S P R F P H T Y P R N T V L V W R L
181 ATTCACAGCC CAAGGTTTCC TCATACTTAT CCAAGAAATA CGGTCTTGGT ATGGAGATTA

+1  V A V E E N V W I Q L T F D E R F G L E
241 GTAGCAGTAG AGGAAAATGT ATGGATACAA CTTACGTTTG ATGAAAGATT TGGGCTTGAA

+1  D P E D D I C K Y D F V E V E E P S D G
301 GACCCAGAAG ATGACATATG CAAGTATGAT TTTGTAGAAG TTGACGAACC CAGTGATGGA

+1  T I L G R W C G S G T V P G K Q I S K G
361 ACTATATTAG GGCCTGGTG TGGTCTGGT ACTGTACCAG GAAAACAGAT TTCTAAAGGA

+1  N Q I R I R F V S D E Y F P S E P G F C
421 AATCAAATTA GGATAAGATT TGTATCTGAT GAATATTTTC CTTCTGAACC AGGGTCTGTC

+1  I H Y N I V M P Q F T E A V S F S V L P
481 ATCCACTACA ACATTGTCAT GCCACAATTC ACAGAAGCTG TGAGTCCTTC AGTGCTACCC

+1  P S A L P L D L L N N A I T A F S T L E
541 CCTTCAGCTT TGCCACTGGA CCTGCTTAAT AATGCTATAA CTGCCTTTAG TACCTTGGAA

+1  D L I R Y L E P E R W Q L D L E D L Y R
601 GACCTTATTC GATATCTTGA ACCAGAGAGA TGGCAGTTGG ACTTAGAAGA TCTATATAGG

+1  P T W Q L L G K A F V F G R K S R V V D
661 CCAACTTGGC AACTTCTTGG CAAGGCTTTT GTTTTGGAA GAAAATCCAG AGTGGTGGAT

+1  L N L L T E E V R L Y S C T P R N F S V
721 CTGAACCTTC TAACAGAGGA GGTAAAGATTA TACAGCTGCA CACCTCGTAA CTTCTCAGTG

+1  S I R E E L K R T D T I F W P G C L L V
781 TCCATAAGGG AAGAACTAAA GAGAACCGAT ACCATTTTCT GGCCAGGTG TCTCCTGGTT

+1  K R C G G N C A C C L H N C N E C Q C V
841 AAACGCTGTG GTGGGAAC TGCCCTGTTGT CTCCACAATT GCAATGAATG TCAATGTGTC

+1  P S K V T K K Y H E V L Q L R P K T G V
901 CCAAGCAAAG TTAATAAAAA ATACCACGAG GTCCTTCAGT TGAGACCAA GACCGGTGTC

+1  R G L H K S L T D V A L E H K E E C D C
961 AGGGGATTGC ACAAATCACT CACCGACGTG GCCCTGGAGC ACCATGAGGA GTGTGACTGT

+1  V C R G S T G G S R Q P F E G K P I P N
1021 GTGTGCAGAG GGAGCACAGG AGGATCTAGA GGGCCCTTCG AAGGTAAAGCC TATCCCTAAC

+1  P L L G L D S T R T C H H H H H H
1081 CCTCTCCTCG GTCTCGATTC TACGCGTACC GGTATCATC ACCATCACCA TTGA

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Figure 20. DNA and polypeptide sequence used for baculovirus/insect cell expression

1 GAATTCAAAG GCCTGTATTT TACTGTTTTT GTAACAGTTT TGTAAATAAA AACCTATAA
+3 m k f l v n v a l v f m v v y i s y i
61 ATATGAAATT CTTAGTCAAC GTTGCCCTTG TTTTATGGT CGTATACATT TCTTACATCT
+3 Y a D P E S H H H H H H E S N L S S K F
121 ATGCGGATCC GGAGTCTCAC CATCACCACC ATCATGAATC CAACCTGAGT AGTAAATTCC
+3 Q F S S N K E Q N G V Q D P Q H E R I I
181 AGTTTTCAG CAACAAGGAA CAGAACGGAG TACAAGATCC TCAGCATGAG AGAATTATTA
+3 T V S T N G S I H S P R F P H T Y P R N
241 CTGTGCTIAC TAAIGGAAGT ATTCACAGCC CAAGGTTTCC TCATACTIAT CCAAGAAATA
+3 T V L V W R L V A V E E N V W I Q L T F
301 CGGTCTTGGT ATGGAGATTA GTAGCAGTAG AGGAAAATGT ATGGATACAA CTTACGTTTG
+3 D E R F G L E D P E D D I C K Y D F V E
361 ATGAAAGATT TGGGCTTGAA GACCCAGAAG ATGACATATG CAAGTATGAT TTTGTAGAAG
+3 V E E P S D G T I L G R W C G S G T V P
421 TTGAGGAACC CAGTGATGGA ACTATATTAG GCGCTGGTG TGGTCTGGT ACTGTACCAG
+3 G K Q I S K G N Q I R I R F V S D E Y F
481 GAAACAGAT TCTAAAGGA AATCAAATTA GGATAAGATT TGTATCTGAT GAATATTTTC
+3 P S E P G F C I H Y N I V M P Q F T E A
541 CTTCTGAACC AAGGTTCTGC ATCCACTACA ACATTGTCAT GCCACAATC ACAGAAGCTG
+3 V S P S V L P P S A L P L D L L N N A I
601 TGAGTCCTTC AATGCTACCC CCTTCAGCTT TGCCACTGGA CTGCTTAAT AATGCTATAA
+3 T A F S T L E D L I R Y L E P E R W Q L
661 CTGCTTTTAG TACCTTGGA GACCTTATC GATATCTTGA ACCAGAGAGA TGGCAGTTGG
+3 D L E D L Y R P T W Q L L G K A F V F G
721 ACTTAGAAGA TTTATATAGG CCAACTTGGC AACTTCTTGG CAAGGCTTTT GTTTTGGAA
+3 R K S R V V D L N L L T E E V R L Y S C
781 GAAATCCAG AATGGTGGAT CTGAACCTTC TAACAGAGGA GGTAAGATTA TACAGCTGCA
+3 T P R N F S V S I R E E L K R T D T I F
841 CACCTCGTAA CTTCTCAGTG TCCATAAGGG AAGAACTAAA GAGAACCGAT ACCATTTTCT
+3 W P G C L L V K R C G G N C A C C L H N
901 GGCCAGGTTG TCTCCTGGTT AACGCTGTG GTGGGAAGT TGCCTGTTGT CTCCACAATT
+3 C N E C Q C V P S K V T K K Y H E V L Q
961 GCAATGAATG TCAATGTGTC CCAAGCAAAG TTAATAAAAA ATACCACGAG GTCCTTCAGT
+3 L R P K T G V R G L H K S L T D V A L E
1021 TGAGACCAAA GACCGGTGTC AGGGGATTGC ACAATCACT CACCGACGTG GCCCTGGAGC
+3 H H E E C D C V C R G S T G G
1081 ACCATGAGGA GTGTGACTGT GTGTGCAGAG GGAGCACAGG AGGATAGCTC TAGA

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Figure 21. DNA and polypeptide sequence used for *E.coli* expression

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+3  Q T N S S S N N N N N N N N N N L G I
1  CGCAGACTAA TTCGAGCTCG AACAAACAACA ACAATAACAA TAACAACAAC CTCGGGGATCG

+3  E G R I S E F E S N L S S K F Q F S S N
61  AGGGAAGGAT TTCAGAATTC GAATCCAACC TGAGTAGTAA ATTCCAGTTT TCCAGCAACA

+3  K E Q N G V Q D P Q H E R I I T V S T N
121 AGGAACAGAA CGGAGTACAA GATCCTCAGC ATGAGAGAAT TATTACTGTG TCTACTAATG

+3  G S I H S P R F P H T Y P R N T V L V W
181 GAAGTATTCA CAGCCCAAGG TTTCTCATA CTTATCCAAG AAATACGGTC TTGGTATGGA

+3  R L V A V E E N V W I Q L T F D E R F G
241 GATTAGTAGC AGTAGAGGAA AATGTATGGA TACAACTTAC GTTGTATGAA AGATTTGGGC

+3  L E D P E D D I C K Y D F V E V E E P S
301 TTGAAGACCC AGAAGATGAC ATATGCAAGT ATGATTTTGT AGAAGTTGAG GAACCCAGTG

+3  D G T I L G R W C G S G T V P G K Q I S
361 ATGGAATAT ATTAGGGCGC TGGTGTGGTT CTGGTACTGT ACCAGGAAAA CAGATTCTTA

+3  K G N Q I R I R F V S D E Y F P S E P G
421 AAGGAAATCA AATTAGGATA AGATTGTAT CTGATGAATA TTTTCTTCT GAACCAAGGT

+3  F C I H Y N I V M P Q F T E A V S P S V
481 TCTGCATCCA CTACACATT GTCATGCCAC AATTCACAGA AGCTGTGAGT CCTTCAGTGC

+3  L P P S A L P L D L L N N A I T A F S T
541 TACCCCTTTC AGCTTTGCCA CTGGACCTGC TTAATAATGC TATAACTGCC TTTAGTACCT

+3  L E D L I R Y L E P E R W Q L D L E D L
601 TGGAGACCT TATTGATAT CTTGAACCAG AGAGATGGCA GTTGGACTTA GAAGATCTAT

+3  Y R P T W Q L L G K A F V F G R K S R V
661 ATAGGCCAAC TTGGCAACTT CTTGGCAAGG CTTTGTGTTT TGGGAAGAAA TCCAGAGTGG

+3  V D L N L L T E E V R L Y S C T P R N F
721 TGGATCTGAA CCTTCTAACA GAGGAGGTAA GATTATACAG CTGCACACCT CGTAACCTCT

+3  S V S I R E E L K R T D T I F W P G C L
781 CAGTGTCAT AAGGAAGAA CTAAAGAGAA CCGATACCAT TTTCTGGCCA GGTGTCTCC

+3  L V K R C G G N C A C C L H N C N E C Q
841 TGGTTAAACG CTGTGGTGGG AACTGTGCCT GTTGTCTCCA CAATGCAAT GAATGTCAAT

+3  C V P S K V T K K Y H E V L Q L R P K T
901 GTGTCCCAAG CAAAGTTACT AAAAAATACC ACGAGGTCCT TCAGTTGAGA CCAAAGACCG

+3  G V R G L H K S L T D V A L E H H E E C
961 GTGTCAAGGG ATTGCACAAA TCACTACCG ACGTGGCCCT GGAGCACCAT GAGGAGTGTG

+3  D C V C R G S T G G H H H H H H *
1021 ACTGTGTGTG CAGAGGGAGC ACAGGAGGAC ATCATCACCA TCACCATTGA TCTAGAGTCG

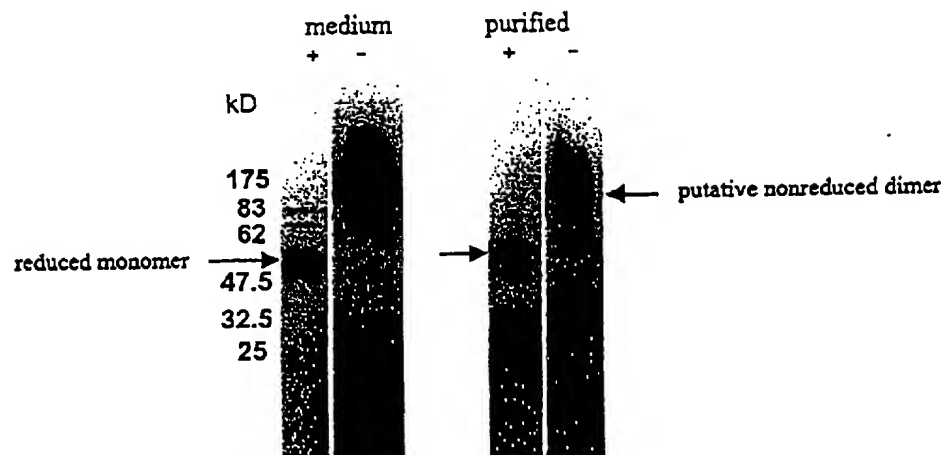
1081 ACCTGCAGGC AAGCTT

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Figure 22. Disulphide-linked dimerisation of VEGF-X

(A) Mammalian cell expression



(B) *E.coli* expression

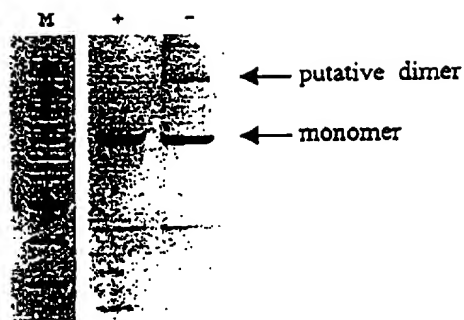
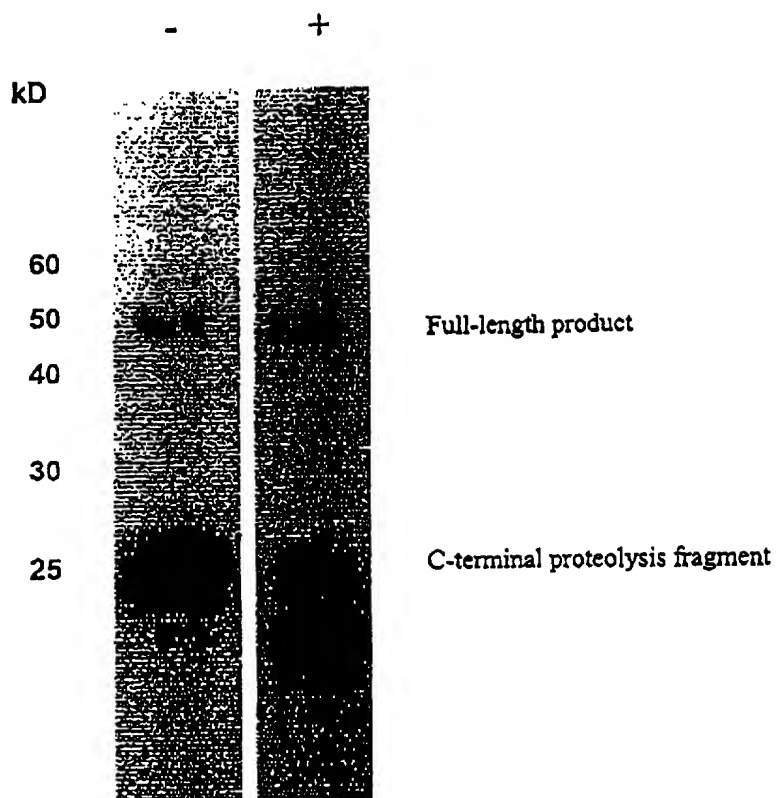


Figure 23. Glycosylation of VEGF-X



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Figure 24. DNA and polypeptide sequence used for *E.coli* expression of the PDGF-like domain

```

+3      M R G S H H H H H H G M A S M
1  AAGGAGATAT ACATATGCGG GGTTCATC ATCATCATCA TCATGGTATG GCTAGCATGA

+3      I G G O O M G R D L Y D D D D K D P G R
61 CTGGTGGACA GCAAATGGGT CGGGATCTGT ACGACGATGA CGATAAGGAT CCGGGAAGAA

+3      K S R V V D L N L L T E E V R L Y S C T
121 AATCCAGAGT GGTGGATCTG AACCTTCTAA CAGAGGAGGT AAGATTATAC AGCTGCACAC

+3      P R N F S V S I R E E L K R T D T I F W
181 CTCGTAACIT CTCAGTGTC ATAAGGGAAG AACTAAAGAG AACCGATACC ATTTCTGGC

+3      P G C L L V K R C G G N C A C C L H N C
241 CAGGTGTCT CCTGGTTAAA CGCTGTGGTG GGAAGTGTGC CTGTTGTCTC CACAATTGCA

+3      N E C Q C V F S K V T K K Y H E V L Q L
301 ATGAATGTCA ATGTGTCCA AGCAAAGTTA CTAAAAATA CCACGAGGTC CTTCASTTGA

+3      R P K T G V R G L H K S L T D V A L E H
361 GACCAAGAC CGGTGTCAGG GGATTGCACA AATCACTCAC CGACGTGGCC CTGGAGCACC

+3      H E E C D C V C R G S T G G
421 ATGAGGAGTG TGAAGTGTG TGCAGAGGGA GCACAGGAGG ATAATGAATT CGAAGCTTGA

481 TCCGGCTGCT AACAAAGCCC

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Figure 25. Expression of PDGF domain in *E.coli*



09463647.122499
667277.4999460

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Figure 26. DNA and polypeptide sequence used for *E.coli* expression of the CUB-like domain

```

+2   M A M D I G I N S D P E S H H H H H H
1   G G C G A T G G C C A T G G A T A T C G A A T T A A T T C G G A T C C G G A G T C T C A C C A T C A C C A C C A T C A

+2   E S N L S S K F Q F S S N K E Q N G V Q
61  T G A A T C C A A C C T G A G T A G T A A A T T C C A G T T T T C C A G C A A C A A G G A A C A G A A C G G A G T A C A

+2   D P Q H E R I I T V S T N G S I H S P R
121 A G A T C C T C A G C A T G A G A G A A T T A T T A C T G T G T C T A C T A A T G G A A G T A T T C A C A G C C C A A G

+2   F P H T Y P R N T V L V W R L V A V E E
181 G T T T C C T C A T A C T T A T C C A A G A A T A C G G T C T T G G T A T G G A G A T T A G T A G C A G T A G A G G A

+2   N V W I Q L T F D E R F G L E D P E D D
241 A A A T G T A T G G A T A C A A C T T A C G T T T G A T G A A A G A T T T G G G C T T G A A G A C C C A G A A G A T G A

+2   I C K Y D F V E V E E P S D G T I L G R
301 C A T A T G C A A G T A T G A T T T T G T A G A A G T T G A G G A A C C C A G T G A T G G A A C T A T A T T A G G G C G

+2   W C G S G T V P G K Q I S K G N Q I R I
361 C T G G T G T G G T T C T G G T A C T G T A C C A G G A A A A C A G A T T T C T A A G G A A A T C A A A T T A G G A T

+2   R F V S D E Y F P S E P G F C I H Y N I
421 A A G A T T T G T A T C T G A T G A A T A T T T C C T T C T G A A C C A G G G T T C T G C A T C C A C T A C A A C A T

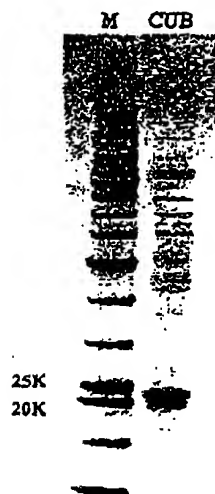
+2   V M P Q F T E A V
481 T G T C A T G C C A C A A T T C A C A G A A G C T G T G T A G T C G A G C T C C G T C G A C A A G C T T G C G G C C C G

541 A C T C G A G C A C

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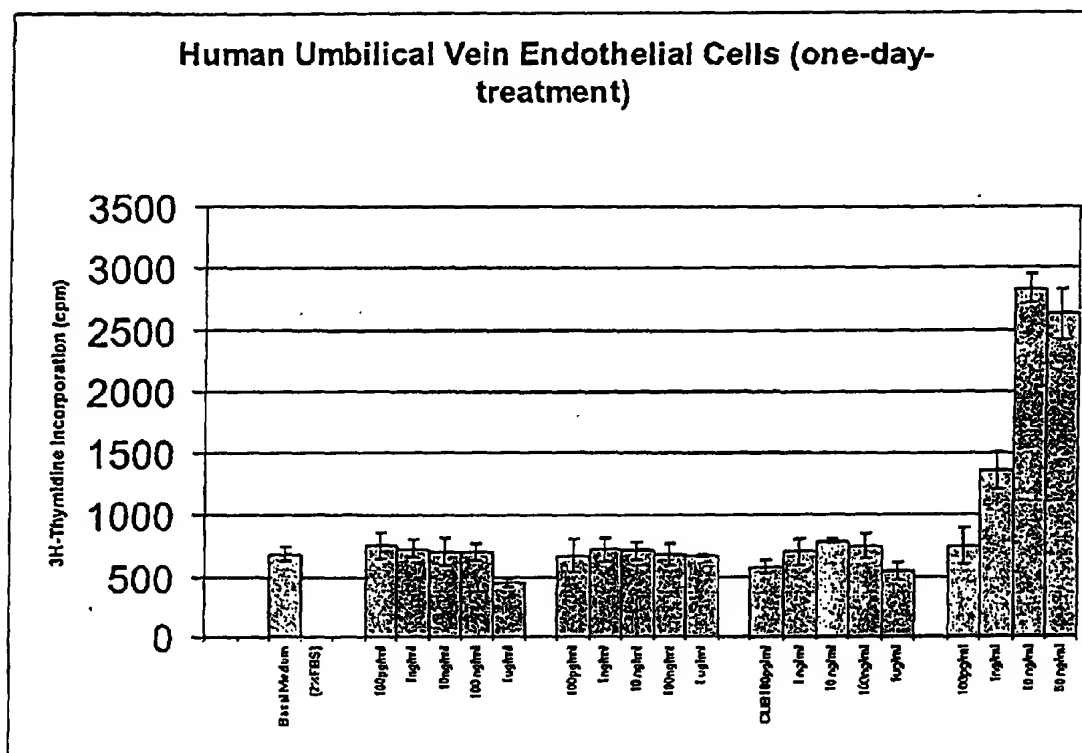
Figure 27. Expression of the CUB domain in *E.coli*



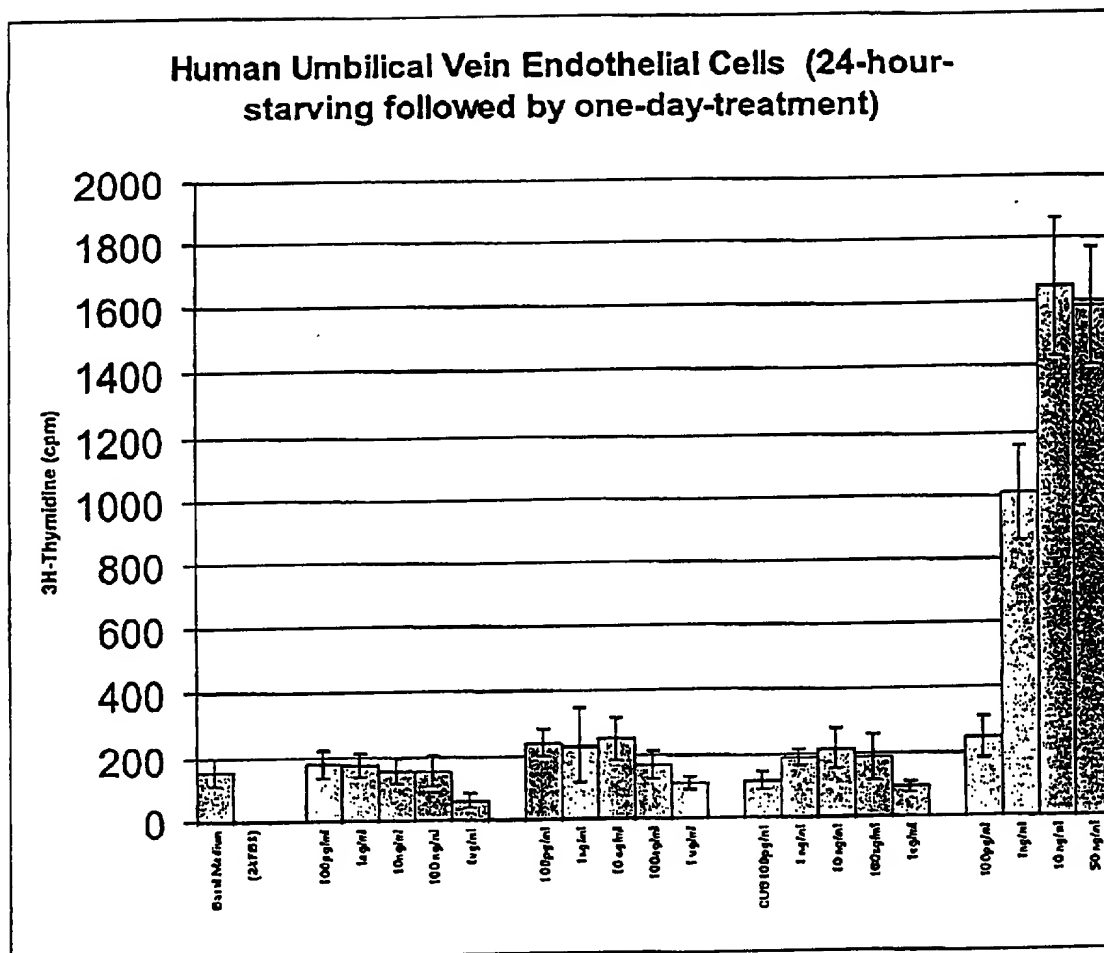
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Figure 28. The Effect of Truncated VEGF-X (CUB domain) on HUVEC Proliferation

(A)



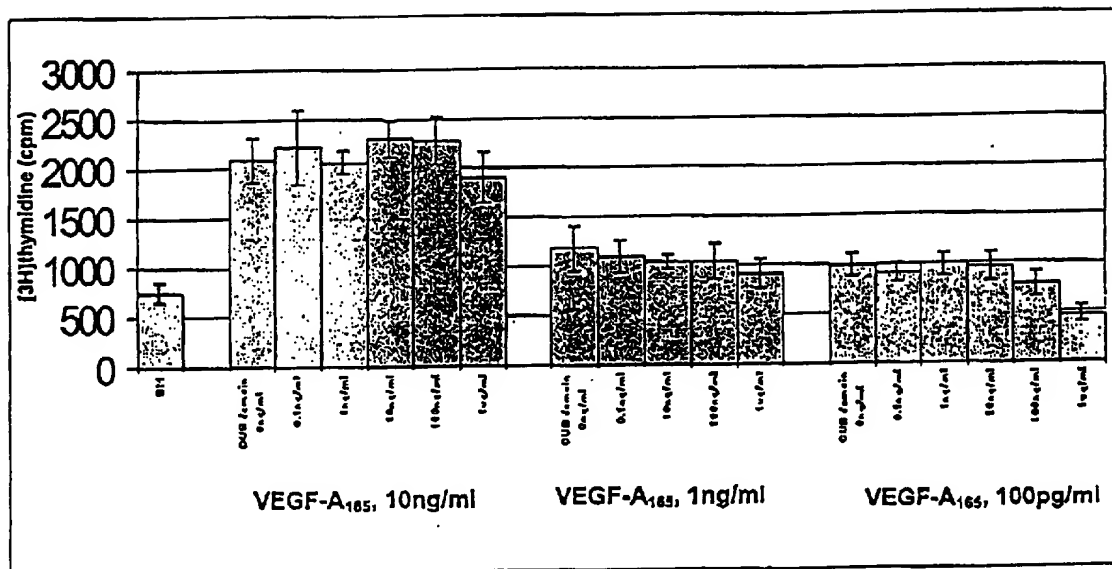
(B)



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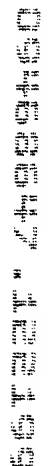
Figure 28

(C)- The effect of VEGF-A₁₆₅ and VEGF-X CUB domain on the proliferation of HUVEC (two-day-treatment).



1. *Staphylococcus aureus* (Staph. aureus) is a common cause of skin infections, such as boils and abscesses. It is also a leading cause of hospital-acquired infections, including pneumonia and bloodstream infections.

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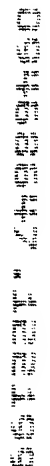


Figure 30. Partial intron/exon structure of the VEGF-X gene

(A) - Genomic DNA sequences of 2 exons determined by sequencing

tttcttttataccatatagtgggtggatctgaaccagGGTTCTGCATCCACTACAACATTGTCATGCCACAATTCACAGAAGCTGTG
AGTCCTTCAGTGCTACCCCTTCAGCTTTGCCACTGGACCTGCTTAATAATGCTATAACTGCCCTTTAGTACCTTGAAGACCTTAT
TCGATATCTTTGAACCAGAGAGATGGCAGTTGGACTTAGAAGATCTATATAGGCCAACTTGGCAACTTCTTGGCAAGGCTTTTGT
TTGGAAGAAAATCCAGAGTGGTGGATCTGAACCTTCTAACAGAGGAGGTAAGATTATACAGCTGCACACCTCGTAACCTTCTCAGTG
TCCATAAGGGAAGAACTAAAGAGAACCATACCATTTTCTGGCCAGGTTGTCTCCTGGTTAAACGCTGTGGTGGGAAGCTGTGCCTG
TTGTCTCCACAATTGCAATGAATGTCAATGTGTCCCAAGCAAAGTTACTAAAAATACCACGAGgtagggtatatacaattttctttt
ggcttctcttcgggtattttatgctt

aaagccagtcataagacattcgttgatttttaaaagtgggttactcttattcccttttcagGTCCTTCAGTTGAGACCAAAGACCGGT
GTCAGGGGATTGCACAAATCACTCACCGACGTGGCCCTGGAGCACCATGAGGAGTGTGACTGTGTGTGCAGAGGGAGCACAGGAGG
ATAGCCGCATCACCACCAGCAGCTCTTGCCAGAGCTGTGCAGTGCAGTGGCTGATTCTATTAGAGAACGTATGCGTTATCTCCAT
CCTTAATCTCAGTTGTTTGCTTCAAGGACCTTTCATCTCAGGATTACAGTGCATTCTGAAAGAGGAGACATCAAACAGAATTAG
GAGTTGTGCAACAGCTCTTTTGAGAGGAGGCTAAAGGACAGGAGAAAAGGTCTTCAATCGTGGAAAGAAAATTAAATGTTGTATT
AAATAGATCACCAGCTAGTTTCAGAGTTACCATGTACGTATTCCTAGCTGGGTCTGTATTTTCAGTTCTTTTCGATACGGCTTAG
GGTAATGTCACTACAGGAAAAAACTGTGCAAGTGAGCACCTGATTCCGTTGCCCTTGGCTTAACTCTAAAGCTCCATGTCCTGGGC
CTAAATCGTATAAAATCTGGATTTTTTTTTTTTTTTTGGCGCATATTCACATATGTAAACCAGAACATTCTATGTACTACAAACC
TGTTTTTTAAAAGGAAGTATGTTGCTATGAATTAACCTTGTGTGCTGATAGGACAGACTGGATTTTTTCATATTTCTTATTAA
AATTTCTGCCATTTAGAAGAAGAGAACTACATTGCTGTTTGGAGAGATAAACCTGAAAAGAAGAGTGGCCTTATCTTCACTTA
TCGATAAGTCAGTTTATTGTTTCATTGTGTACATTTTATATTCTCCTTTTGACATTATAACTGTGGCTTTTCTAATCTTGTTA
AATATATCTATTTTACCAAGGTATTTAATATTCTTTTTATGACAACTTAGATCAACTATTTTTCAGCTGGTAAATTTTCTAA
ACACAATTGTTATAGCCAGAGGAACAAGATGATATAAAATATTGTTGCTCTGACAAAAATACATGTATTTTCATCTCGTATGGTG
CTAGAGTTAGATTAATCTGCATTTTAAAAAACTGAATTGGAATAGAATTGGTAAGTTGCAAAGACTTTTTGAAAATAATTAAATTA
TCATATCTTCCATTCTGTTATTGGAGATGAAAATAAAAGCAACTTATGAAAGTAGACATTCAGATCCAGCCATTACTAACCTAT
TCCTTTTTTGGGGAATCTGAGCCTAGCTCAGAAAAACATAAAGCACCTTGAAAAGACTTGGCAGCTTCTGTATAAAGCGTGTG
TGCTGTGCAGTAGGAACACATCCTATTTATTGTGATGTTGTGGTTTTATTATCTTAAACTCTGTTCCATACACTTGTATAAATACA
TGGATATTTTATGTACAGAAGTATGTCTTAAACAGTTCACCTATTGTACTCTGGCAATTTAAAAGAAAATCAGTAAATATTT
TGCTTGTAATAATGCTTAATATCGTGCCTAGGTTATGTGGTGACTATTTGAATCAAAAATGTATTGAATCATCAAAATAAAGAAATGT
GGCTATTTTGGGGAGAAAATtatgtgtgtgtgtgtcgaagattttcttggactctgagaaatgaaagataaa

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Figure 30 continued

(B) - Location of splice sites within the cDNA sequence

1 GAATTCGCCC TTTTGTAA ACCTTGGGAA CTGGTTCAGG TCCAGGTTTT GCTTTGATCC
61 TTTTCAAAAA CTGGAGACAC AGAAGAGGGC TCTAGGAAAA AGTTTGGAT GGGATTATGT
121 GGAAACTACC CTGCGATTCT CTGCTGCCAG AGCAGGCTCG GCGCTTCCAC CCCAGTGCAG
181 CCTTCCCCTG GCGGTGGTGA AAGAGACTCG GGAGTCGCTG CTTCCAAAGT GCGCGCCGTG
+3 M S L F G L L L L T S
241 AGTGAGCTCT CACCCAGTC AGCCAAATGA GCCTCTTCGG GCTTCTCCTG CTGACATCTG
+3 A L A G Q R Q G T Q A E S N L S S K F Q
301 CCCTGGCCGG CCAGAGACAG GGGACTCAGG CGGAATCCAA CCTGAGTAGT AAATCCAGT
+3 F S S N K E Q N G V Q D P Q H E R I I T
361 TTTCCAGCAA CAAGGAACAG AACGGAGTAC AAGATCCTCA GCATGAGAGA ATTATTACTG
+3 V S T N G S I H S P R F P H T Y P R N T
421 TGCTACTAA TGGAAGTATT CACAGCCCAA GGTTTCCTCA TACTTATCCA AGAAATACGG
+3 V L V W R L V A V E E N V W I Q L T F D
481 TCTTGGTATG GAGATTAGTA GCAGTAGAGG AAAATGTATG GATACAACTT ACGTTTGATG
+3 E R F G L E D P E D D I C K Y D F V E V
541 AAAGATTGG GCTTGAAGAC CCAGAAGATG ACATATGCAA GTATGATTTT GTAGAAGTTG
+3 E E P S D G T I L G R W C G S G T V P G
601 AGGAACCCAG TGATGGAAC TATTAGGGC GCTGGTGTGG TTCTGGTACT GTACCAGGAA
+3 K Q I S K G N Q I R I R F V S D E Y F P
661 AACAGATTTC TAAAGGAAAT CAAATTAGGA TAAGATTGT ATCTGATGAA TATTTTCCTT
+3 S E P G F C I H Y N I V M P Q F T E A V
721 CTGAACCAAG GTTCTGCATC CACTACAACA TTGTCATGCC ACAATCACA GAAGCTGTGA
+3 S P S V L P P S A L P L D L L N N A I T
781 GTCCTTCAGT GCTACCCCTC TCAGCTTGC CACTGGACCT GCTTAATAAT GCTATAACTG
+3 A F S T L E D L I R Y L E P E R W Q L D
841 CCTTTAGTAC CTGGAAGAC CTTATTCGAT ATCTTGAACC AGAGAGATGG CAGTTGGACT
+3 L E D L Y R P T W Q L L G K A F V F G R
901 TAGAAGATCT ATATAGGCCA ACTTGGCAAC TTCITGGCAA GGCITTTGTT TTTGGAAGAA
+3 K S R V V D L N L L T E E V R L Y S C T
961 AATCCAGAGT GGTGGATCTG AACCTTCTAA CAGAGGAGT AAGATTATAC AGCTGCACAC
+3 P R N F S V S I R E E L K R T D T I F W
1021 CTCGTAACTT CTCAGTGTCC ATAAGGGAAG AACTAAAGAG AACCGATACC ATTTTCTGGC
+3 P G C L L V K R C G G N C A C C L H N C
1081 CAGGTTGTCT CTTGGTTAAA CGCTGTGGTG GGAAGTGTGC CTGTTGTCTC CACAATTGCA
+3 N E C Q C V P S K V T K K Y H E V L Q L
1141 ATGAATGTCA ATGTGTCCCA AGCAAAGTTA CTAAAAATA CCACGAGTCT CTTCAAGTTG

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+3 R P K T G V R G L H K S L T D V A L E H
 1201 GACCAAAGAC CGGTGTCAGG GGATTGCACA AATCACTCAC CGACGTGGCC CTGGAGCACC
 +3 H E E C D C V C R G S T G G
 1261 ATGAGGAGTG TGACTGTGTG TGCAGAGGGA GCACAGGAGG ATAGCCGCAT CACCACCAGC
 1321 AGCTCTTGCC CAGAGCTGTG CAGTGCAGTG GCTGATTCTA TTAGAGAACG TATGCGTTAT
 1381 CTCCATCCTT AATCTCAGTT GTTGTCTCA AGGACCTTC ATCTTCAGGA TTTACAGTGC
 1441 ATTCTGAAAG AGGAGACATC AAACAGAATT AGGAGTTGTG CAACAGCTCT TTTGAGAGGA
 1501 GGCCTAAAGG ACAGGAGAAA AGGTCTTCAA TCGTGGAAG AAAATTAAAT GTTGTATTAA
 1561 ATAGATCACC AGCTAGTTTC AGAGTTACCA TGTACGTATT CCACTAGCTG GGTTCGTAT
 1621 TTCAGTTCTT TCGATACGGC TTAGGGTAAT GTCAGTACAG GAAAAAACT GTGCAAGTGA
 1681 GCACCTGATT CCGTTGCCTT GCTTAACTCT AAAGCTCCAT GTCCTGGGCC TAAAATCGTA
 1741 TAAAATCTGG ATTTTTTTTT TTTTTTTTG CTCATATTCA CATATGTAAA CCAGAACATT
 1801 CTATGTACTA CAAACCTGGT TTTTAAAAG GAACTATGTT GCTATGAATT AAACCTGTGT
 1861 CATGCTGATA GGACAGACTG GATTTTTCAT ATTTCTTATT AAAATTTCTG CCATTTAGAA
 1921 GAAGAGAACT ACATTATGG TTTGGAAGAG ATAAACCTGA AAAGAAGAGT GGCCTTATCT
 1981 TCACTTTATC GATAAGTCAG TTTATTGTT TCATTGTGTA CATTTTATA TTCTCCTTTT
 2041 GACATTATAA CTGTTGGCTT TTCTAATCTT GTTAAATATA TCTATTTTTA CCAAAGGTAT
 2101 TTAATATTCT TTTTATGAC AACTTAGATC AACTATTTT AGCTTGGTAA ATTTTCTAA
 2161 ACACAATTGT TATAGCCAGA GGAACAAAGA TGATATAAAA TATTGTTGCT CTGACAAAAA
 2221 TACATGTATT TCATTCTCGT ATGGTGCTAG AGTTAGATTA ATCTGCATT TAAAAAACTG
 2281 AATTGGAATA GAATTGGTAA GTTGCAAAGA CTTTTTGAAA ATAATTAAAT TATCATATCT
 2341 TCCATTCTCG TTATTGGAGA TGAAAATAAA AAGCAACTTA TGAAAGTAGA CATTAGATC
 2401 CAGCCATTAC TAACCTATTC CTTTTTTGGG GAAATCTGAG CCTAGCTCAG AAAACATAA
 2461 AGCACCTTGA AAAAGACTTG GCAGCTTCCT GATAAAGCGT GCTGTGCTGT GCAGTAGGAA
 2521 CACATCCTAT TTATTGTGAT GTTGTGGTTT TATTATCTTA AACTCTGTTT CATACACTTG
 2581 TATAAATACA TGGATATTTT TATGTACAGA AGTATGTCTC TTAACCAGTT CACTTATTGT
 2641 ACCTGGAAGG GCGAATTCTG CAGATATC

Fig. 30 (cont.)

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The Effect of FL-VEGF-X on HUVEC Proliferation: (24-hour serum starvation followed by one day- treatment)

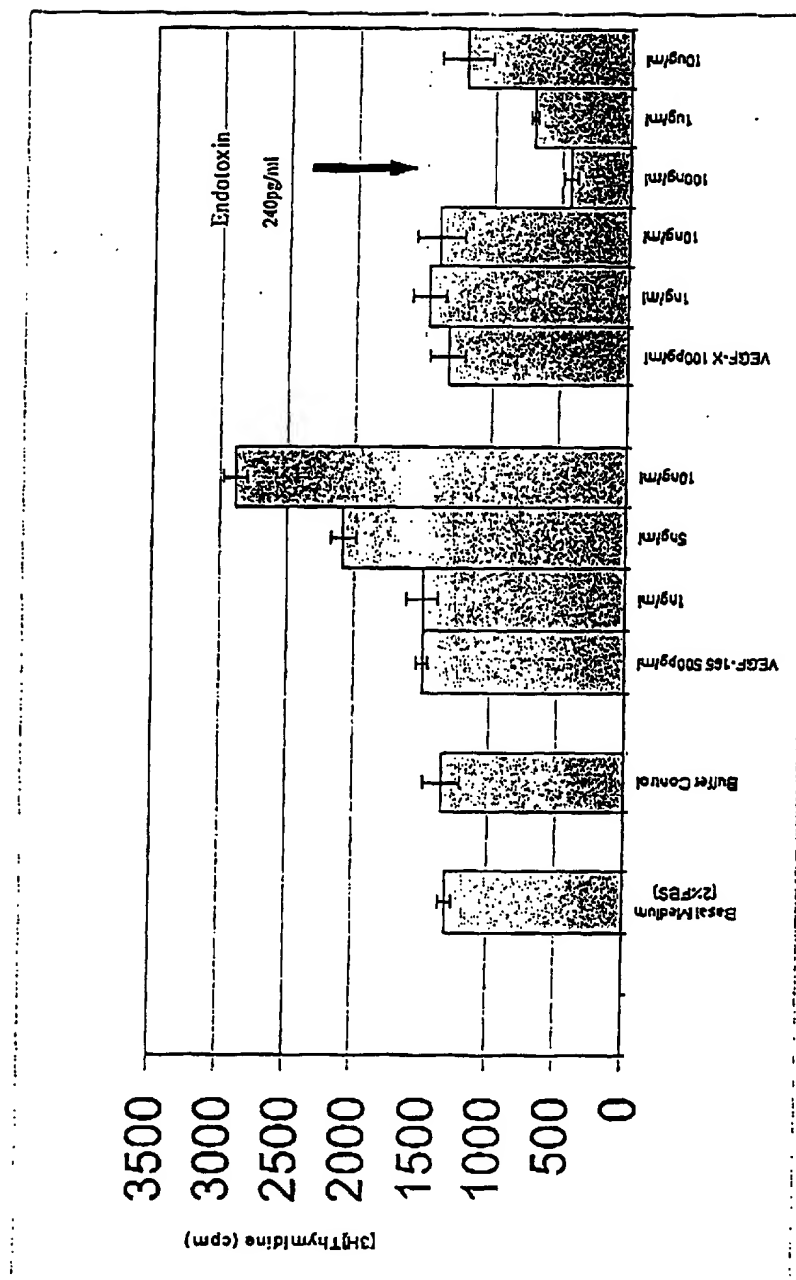


Fig. 31

The Combined Effect of Truncated VEGF-X (CUB domain) and Human Recombinant VEGF₁₆₅ on HUVEC Proliferation: (24-hour serum starvation followed by two-day-treatment)

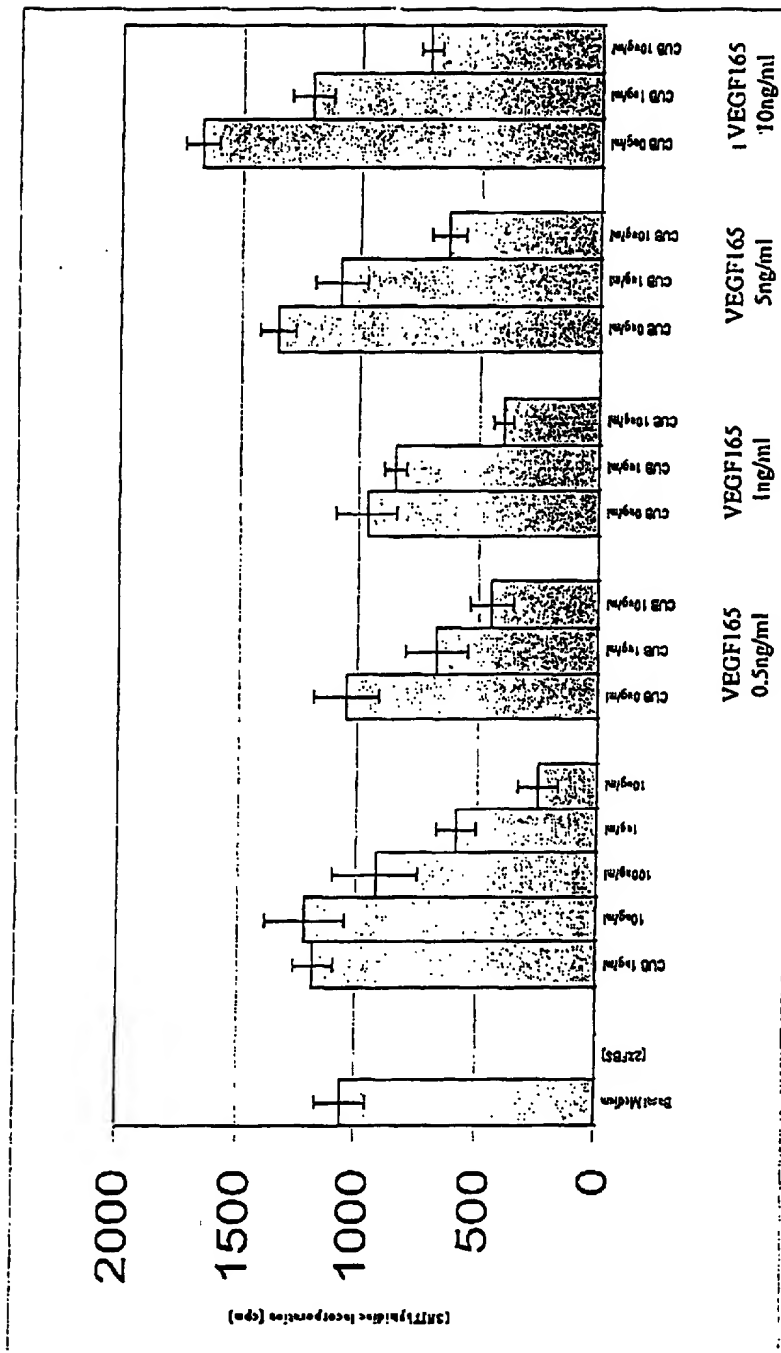


Fig. 32

LDH Assay for Testing Cytotoxicity of CUB Domain or CUB Domain with rhVEGF₁₆₅

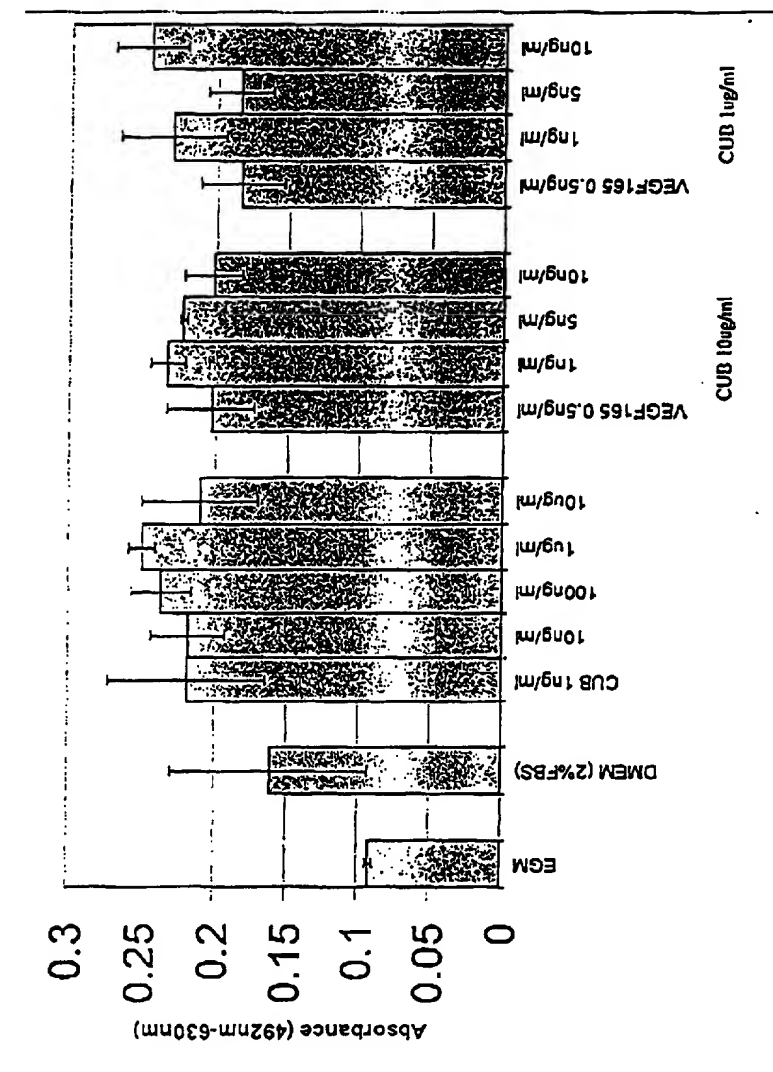


Fig 34

LDH Assay for Testing Cytotoxicity of CUB Domain or CUB Domain with rh-bFGF

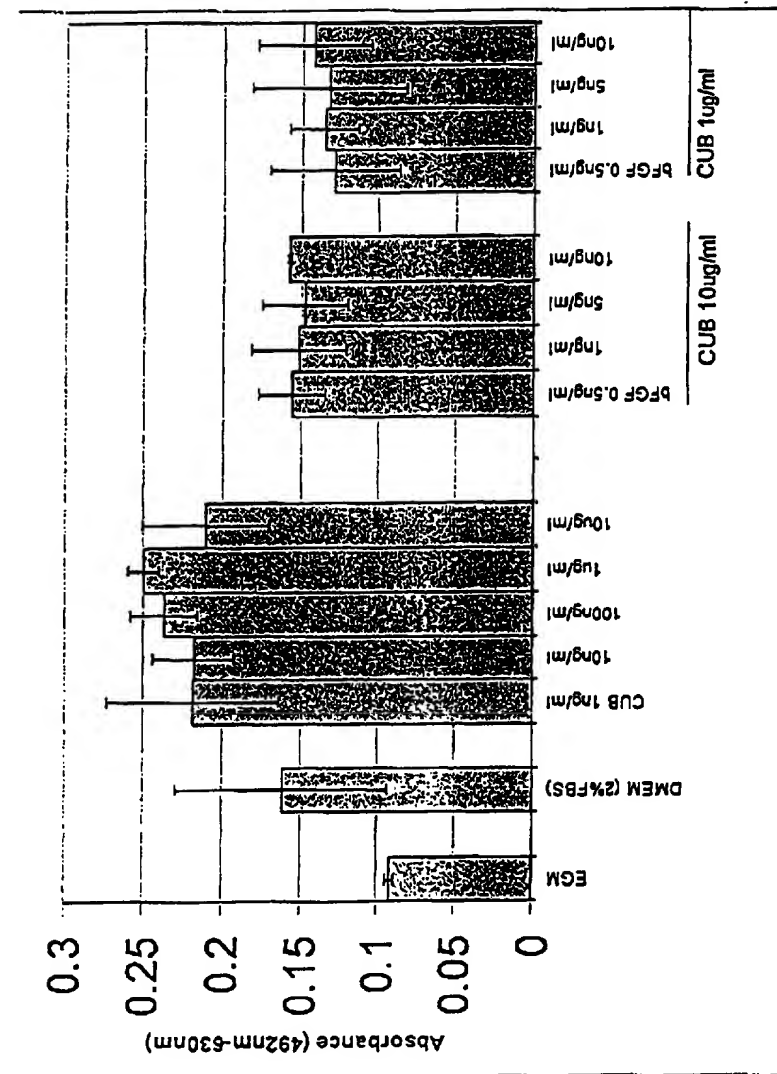


Fig 35